ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECTS OF PRETREATMENT METHODS ON LIGNOCELLULOSIC BIOETHANOL PRODUCTION

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

LİGNOSELÜLOZİK BİYOETANOL ÜRETİMİNDE ÖNİŞLEMLERİN ETKİSİ

YÜKSEK LİSANS TEZİ

Merve Nazlı ERDÖNMEZ BORAND
(506091045)

Kimya Mühendisliği Anabilim Dalı

Kimya Mühendisliği Programı

Tez Danışmanı: Prof. Dr. Filiz KARAOSMANOĞLU

OCAK 2012

Merve Nazlı ERDÖNMEZ BORAND, a **M.Sc..** student of ITU **Graduate School of Science, Engineering and Technology** student ID **506091045**, successfully defended the **thesis** entitled **"Effects of Pretreatment Methods on Lignocellulosic Bioethanol Production"**, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Filiz KARAOSMANOĞLU** İstanbul Technical University	.....
Jury Members :	**Prof. Dr. Güldem ÜSTÜN** İstanbul Technical University	.....
	Prof. Dr. Name SURNAME Yıldız Technical University	.....

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FOREWORD

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December 2011 Merve Nazlı ERDÖNMEZ BORAND

Chemical Engineer

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ABBREVIATIONS

AFEX : Ammonia Fiber Explosion
ARP : Ammonia Recycling Percolation
ASTM : American Society for Testing and Materials
BM : Ball Mill Pretreatment
BM35 : 35 mesh size of sawdust with ball mill pretreatment
BM35-H : 35 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM42 : 42 mesh size of sawdust with ball mill pretreatment
BM42-H : 42 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM65 : 65 mesh size of sawdust with ball mill pretreatment
BM65-H : 65 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM75 : 75 mesh size of sawdust with ball mill pretreatment
BM75-H : 75 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM100 : 100 mesh size of sawdust with ball mill pretreatment
BM100-H : 100 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM115 : 115 mesh size of sawdust with ball mill pretreatment
BM115-H : 115 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BM125 : 125 mesh size of sawdust with ball mill pretreatment
BM125-H : 125 mesh size of sawdust with ball mill pretreatment and enzymatic hydrolysis
BtL : Biomass to Liquid
C : Carbon
C_2H_5OH : Ethanol
C5 : 5 Carbon Sugars
C6 : 6 Carbon Sugars
CO_2 : Carbon Dioxide
DI : Deionized water
DNS : Dinitrosalicylic Asid
E10 : 10% Ethanol and 90% gasoline blend
E20 : 20% Ethanol and 80% gasoline blend
E25 : 25% Ethanol and 75% gasoline blend
E85 : 85% Ethanol and 15% gasoline blend
EPDK : Energy Marketing Regulatory Authority
EU : European Union
EtOH : Ethanol
FPA : Filter Paper Activity
FPU : Filter Paper Unit
FT : Fischer - Tropsch
H : Hydrogen

HCl : Hydrochloric acid
HPLC : High Performance Liquid Chromatography
H_2SO_4 : Sulfuric acid
ILs : Ionic Liquids
IUPAC : International Union of Pure and Applied Chemistry
JML : James Martin Laboratory
kcal : Kilocalorie
kg : Kilogram
LHW : Liquid Hot Water
mL : milliliter
mM : Millimolar
mmHg : Millimeter Mercury
mg : Milligram
MPa : Mega Pascal
N : Negative Control
NREL : National Renewable Energy Laboratory
PCR : Polymerase Chain Reaction
psig : Pounds per Square Inch (Gage Pressure)
R&D : Research and Development
rpm : Revolutions per Minute
S1L : Liquid Part of 1st SE Experiment
S1V : Samples from vessel at 1st SE Experiment
S1V-H : Samples from vessel at 1st SE Experiment then enzymatic hydrolysis
S19L : Average of S1L and S9L
S19R : Average of S1R and S9R
S19V : Average of S1V and S9V
S19V-H : Average of S1V-H and S9V-H
S2V-H : Samples from vessel at 2nd SE Experiment then enzymatic hydrolysis
S2L : Liquid Part of 2nd SE Experiment
S237L : Average of S2L, S3L and S7L
S237R : Average of S2R, S3R and S7R
S237R-H : Average of S2R-H, S3R-H and S7R-H
S237V : Average of S2V, S3V and S7V
S237V-H : Average of S2V-H, S3V-H and S7V-H
S3L : Liquid Part of 3rd SE Experiment
S3R-H : Samples from reactor at 3rd SE Experiment then enzymatic hydrolysis
S3V : Samples from vessel at 3rd SE Experiment
S3V-H : Samples from vessel at 3rd SE Experiment then enzymatic hydrolysis
S5L : Liquid Part of 5th SE Experiment
S5R : Samples from reactor at 5th SE Experiment
S5R-H : Samples from reactor at 5th SE Experiment then enzymatic hydrolysis
S5V : Samples from vessel at 5th SE Experiment
S5V-H : Samples from vessel at 5th SE Experiment then enzymatic hydrolysis
S6L : Liquid Part of 6th SE Experiment
S6R : Samples from reactor at 6th SE Experiment
S6R-H : Samples from reactor at 6th SE Experiment then enzymatic hydrolysis
S6V : Samples from vessel at 6th SE Experiment

S6V-H : Samples from vessel at 6th SE Experiment then enzymatic hydrolysis
S7R : Samples from reactor at 7th SE Experiment
S7R-H : Samples from reactor at 7th SE Experiment then enzymatic hydrolysis
S7V : Samples from vessel at 7th SE Experiment
S7V-H : Samples from vessel at 7th SE Experiment then enzymatic hydrolysis
S8L : Liquid Part of 8th SE Experiment
S8R : Samples from reactor at 8th SE Experiment
S8V : Samples from vessel at 8th SE Experiment
S9L : Liquid Part of 9th SE Experiment
S9R : Samples from reactor at 9th SE Experiment
S9V : Samples from vessel at 9th SE Experiment
S10L : Liquid Part of 10th SE Experiment
S10R : Samples from reactor at 10th SE Experiment
S10V : Samples from vessel at 10th SE Experiment
SD : Untreated sawdust
SDH : Sawdust after hydrolysis
SE : Steam Explosion
sec : Second
SPORL : Sulfite Pretreatment o Overcome Recalcitrance of Lignocellulose
SSF : Simultaneous Saccharification and Fermentation
T : Temperature
t : Time
TAPDK : Tobacco and Alcohol Market Regulatory Authority
TFA : Trifluoracetic Acids
UI : University of Idaho
UK : United Kingdom
UNICA : Brazilian Sugarcane Industry Association
USA : United States of America
WDM : Wet Disk Milling
WIS : Water Insoluble Solids
wt : Weight

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EFFECTS OF PRETREATMENT METHODS ON LIGNOCELLULOSIC BIOETHANOL PRODUCTION

SUMMARY

In present, bioethanol is leader engine biofuels. Also known as fuel alcohol, bioethanol both used as an alternative to gasoline and used as additive for fuels. First generation bioethanol is produced from sugary and starchy biomass sources. In present, majority of bioethanol production is provided from sugary and starch biomass. Second generation bioethanol, except agricultural products, is produced from enzymatic hydrolysis of lignocellulosic biomass. Lignocellulosic biomass which comprised from wood, agricultural and forestry residues includes high amount of cellulose, hemicellulose and lignin. Cellulose is long polymeric chains which consist of glucose monomers and cellulose is fundamental raw material of bioethanol production. Hemicellulose is polymeric structures which consist of 5 and 6 carbon sugars and it is converted to bioethanol with high ratio. Lignin is chemical compound which support structural stability to plants. Lignin which exist in structure of plant prevent to sugar conversion in enzymatic hydrolysis. For efficiency bioethanol production, physical, chemical, biological, pulsed electric fields and physicochemical pretreatment methods are applied in process. Pretreatment methods cause to break lignin structure, make cellulose and hemicellulose structure more accessible to enzymes.

In this study, as a raw material North Pine Tree which is harvested in 2010, is used for bioethanol production, the effects of ball mill and steam explosion on lignocellulosic biomass to sugar effiency was investigate. Accordingly, pine tree which is used as raw material was sorted by sizing, according to ASTM standards ash, moisture and lignin anaylsis were done. Analysed biomass was milled with ball mill and steam explosion was applied. Samples were hydrolyzed with enzyme and cellulase activity was measured. On the other hand, hydrolyzed and non-hydrolyzed samples were analyzed in High Performenced Liquid Chramotography (HPLC) for determine sugars. According to this study conditions, 75 mesh sized pine particles were chosed after ball mill pretreatment. Then these particles exploded with steam which was at 190°C with 64 psig nitrogen for 10 minutes retention time and sudden releasing has higher sugar conversion.

LİGNOSELÜLOZİK BİYOETANOL ÜRETİMİNDE ÖNİŞLEMLERİN ETKİSİ

ÖZET

1970'li yıllardan önce enerjinin temininde hiçbir problem yokken, 1973'te Petrol Krizi'nin çıkması sonucu ülkelerin petrol sağlayamaması ile birlikte ülkeler enerji konusunda dış kaynaklara bağımlı olmamaları gerektiğini anlamışlardır. Bununla birlikte fosil kaynak rezervlerinin azalması, enerji fiyatlarının yükselmesi, ekolojik dengenin bozulması devletleri alternatif enerji kaynakları aramaya yöneltmiştir. Alternatif kaynaklar arasında yenilenebilir enerji kaynakları ve nükleer enerji sayılabilir. Alternatif enerji kaynakları arasında biyokütle önemli bir yer tutmaktadır. Biyokütlenin diğer enerji kaynaklarından daha üstün olan tarafı, kolay ulaşılabilmesidir yani biyokütle yeryüzünün her yerinde değişik türlerde bulunabilir. Böylelikle biyokütlenin kullanılması ülkelerin enerji ihtiyacında dışa bağımlılığı azaltmakta, enerji güvenliği oluşturmaktadır. Bunun yanı sıra biyokütlenin sürekli olarak hazır bulunabilmesi, biyokütleyi diğer yenilenebilir enerji kaynakları arasında daha öne çıkarmaktadır.

Biyokütle eski çağlardan beri enerji kaynağı olarak kullanılmaktadır. Biyokütlenin geleneksel kullanımı doğrudan yakma şeklindedir fakat biyokütlenin doğrudan yakılması içerdiği nem miktarı nedeniyle verimli bir yanma sağlamamaktadır. Bu nedenle biyokütleden yüksek verimde enerji elde edilebilmesinin sağlanması için biyorafineriler tasarlanmıştır. Biyorafineriler, petrol rafinerilerinden farklı olarak hammadde olarak biokütle kullanmaktadır. Termokimyasal ve biyokimyasal işlemler sonucunda biyokütle, biyokimyasallara, biyomalzemelere ve biyoyakıtlara dönüştürülmektedir. Günümüz biyorafinerileri yeşil biyorafinerileri olarak bilinirler ve gıda maddesi olan biyokütlelerin dönüşümünü sağlarlar. Beyaz biyorafineriler, gıda ile rekabet etmeyen lignoselülozik kaynaklar ve atıkların kullanılmasını sağlayarak toplum ve çevre açısından çok önemlidirler. Biyorafinerilerden üretilen en önemli ürünlerden bir tanesi biyoyakıtlardır.

Biyoyakıtlar, 4 grupta incelenebilirler. Birinci nesil biyoyakıtlar, hammadde olarak gıda maddesi olarak da kullanılan tarım ürünlerinden elde edilmektedir. Günümüz teknolojisi ile yağlardan üretilen biyodizel ve şeker ve nişastalı besinlerden üretilen biyoetanol bu grup içerisindedir. İkinci nesil biyoyakıtlar atık yağlardan ve lignoselülozik biyokütleden üretilmektedir. Lignoselülozik biyoetanol ikinci kuşak biyoyakıtlar arasında yer almaktadır. Birinci nesil biyoyakıtlardan farkı, hammaddeleri gıda ürünü olarak kullanılmamaktadır ve böylece gıda sektörü ile rekabet halinde değildir. İkinci nesil biyoyakıtlar günümüzde üretilmeye başlanmıştır fakat ticari özelliğinin artması için çalışmaların yapılması gerekmektedir. Üçüncü nesil biyoyakıtlar, genetiği değiştirilmiş bitki ve alglerden oluşmaktadır. Genetik değişim sonrası, yapılarında bulunan yağ ve selüloz miktarı arttırılmaktadır. Dördüncü nesil biyoyakıtlar, karbon negatif biyoyakıtlar olarak da bilinirler. Genetiği

mükemmelleştirilmiş bitki ve algler tarafından üretilmektedir. Karbon negatif özelliği, enerji açığa çıkartırken oluşturduğu karbondioksiti besin olarak kullanabilmesi sonucu net karbondioksit salınımının sıfır olmasından kaynaklanmaktadır. Üçüncü ve dördüncü nesil biyoyakıtlar gelecek yıllarda yakıt üretiminde çok önemli rol oynayacaklardır fakat günümüzde hala araştırma aşamasında bulunmaktadırlar.

Biyoetanol, günümüzün lider motor biyoyakıtıdır. Yakıt alkolü olarak da bilinen biyoetanol hem benzine alternatif hem de yakıt katkı maddesi olarak kullanılmaktadır. Günümüzde benzine alternatif kullanılan araçların kullanılmasıyla birlikte, benzine katkı maddesi olarak kullanımı daha yaygındır ve birçok ülkede yasalar ile zorunlu hale getirilmiştir. 2010 verilerine göre Dünya'daki biyoetanol üretimi yaklaşık 100 milyar litre iken Türkiye'deki üretim 184 milyon litredir. Dünya'da biyoetanol üretiminde lider ülke ABD iken daha sonra Brezilya en fazla biyoetanol üreten ikinci ülkedir.

Birinci kuşak biyoetanol şekerli ve nişastalı biyokütle kaynaklarından üretilmektedir. Günümüz biyoetanol üretiminin büyük çoğunluğu şekerli ve nişastalı biyokütlelerden sağlanmaktadır. Birinci kuşak biyoetanol üretiminde en çok kullanılan hammaddeler şeker pancarı, şeker kamışı, mısır, buğday ve diğer nişastalı bitkilerdir. İkinci kuşak biyoetanol tarım kaynakları dışında, lignoselülozik biyokütleden enzimatik hidroliz yöntemi ile üretilmektedir. Odun, tarımsal ve orman atıklarından oluşan lignoselülozik biyokütle büyük oranda selüloz, hemiselüloz ve lignin içermektedir. Selüloz glikoz monomerlerinden oluşan uzun zincir yapıdaki bir polimerdir ve biyoetanol üretiminin temel hammaddesidir. Hemiselüloz 5 ve 6 karbonlu şekerlerden oluşan polimerik bir yapıdır ve büyük oranda biyoetanole çevrilebilir. Hemiselüloz yapısındaki 5 karbonlu şekerlere örnek olarak galaktoz, mannoz, arabinose, xyloz verilebilir. 6 karbonlu şeker ise glukozdur. Lignin ise bitkiye yapısal destek sağlayan kimyasal bileşimdir. Enzimatik hidrolizde yapıdaki lignin şeker oluşumu engellemektedir ve bu nedenle biyoetanol üretiminde istenmemektedir. Biyodönüşüm yönteminde, hammadde olarak kullanılan lignoselülozik biyokütlenin içerdiği lignin yapısının kırılması ve enzimin selüloza daha kolay erişebilmesi için önişlemler uygulanır. Verimli bir biyoetanol üretimi için lignoselülozik biyokütleye fiziksel, kimyasal, biyolojik, atım elektrik alan ve fizikokimyasal önişlemlerin uygulanması gerekmektedir. Mekanik püskürtme (ezme, öğütme) ve ekstruder fiziksel önişlemlere örnek olarak gösterilebilir. Kimyasal önişlemler ise asit, baz ve iyonik sıvılar önişlem için kullanılmaktadır. Fizikokimyasal önişlemde ise AFEX, karbondioksit uygulama, ıslak oksidasyon, mikrodalga uygulaması, ultrasound uygulaması, süperkritik sıvılar uygulaması ve buharda patlatma gibi yöntemler uygulanmaktadır. Önişlemler, lignoselülozik yapının kırılmasını ve enzimin selüloz ve hemiselüloz yapıya daha kolay ulaşmasını sağlamaktadır. Önişlem uygulanmış biyokütle enzim ile hidrolize edilerek içerdiği selüloz ve hemiselülozun, 5 ve 6 karbonlu şekerlere dönüşümü sağlanır. Oluşan şeker solüsyonu, fermantasyon yöntemi ile etanole çevrilir. Fermantasyon için bakteri ve mayalar kullanılmaktadır ve 5 ve 6 karbonlu şekerler için kullanılan mayalar ve bakteriler farklıdır. Teorik olarak fermantasyon verimi %95 civarındadır. Fermantasyon sonucu çıkan karışımın etanol oranı % 7 - 7,5 arasındadır. Distilasyon ve susuzlaştırma işlemleri sonucunda bu oran yükseltilerek kullanıma hazır hale getirilir. Biyodönüşümün yanı sıra etanol üretiminde termokimyasal yöntemler de uygulanmaktadır fakat bu çalışmada yer verilmemektedir.

Bu çalışmanın amacı, lignoselülozik biyokütlenin değişik önişlemler sonucunda şekere dönüşüm verimlerinin ölçülmesidir. Bu amaçla, mekanik püskürtme yöntemlerinden bilyalı değirmen ve fizikokimyasal önişlemlerden de buharda patlatma yöntemi, önişlem olarak seçilmiştir. Bilyalı değirmen önişleminde temel prensip, lignoselülozik biyokütlenin parça büyüklüğünün azaltılması, alanının arttırılması, kristalin yapısının bozulması, içerisindeki polimerik zincir yapının daha basit şekerlere dönüşümünün sağlanmasıdır. Bu önişlem için lignoselülozik biyokütle bilyalı değirmende değişik sürelerde öğütülmüştür. Buharda patlatma işleminde ise parçacıklar önce yüksek sıcaklık ve basınçta buhar ile muamele edilir. Bu basınç aniden atmosfer basıncına düşürülür ve böylece basınç etkileşimi ile lignoselülozik parçaların fiziksel yapısı değişir. Buharda patlatma yönteminde, lignoselülozik biyokütlenin fiziksel yapısı değişmekte, kristalin yapısı bozulmaktadır.

Bu çalışmada, hammadde olarak 2010 yılı kesimi Kuzey Amerika çam ağacı kullanılmıştır. Çam ağacının kullanılma nedeni reçine içeriği düşük olan sert olmayan bir lignoselülozik biyokütle türü olmasıdır. Soyulmuş odun halinde alınan çam örneği önce parçalanmış daha sonra da daha küçük parçacıklar halinde doğranmıştır ve parça büyüklüklerine göre 7 gruba ayrılmıştır. Gruplara ayrılmada elek kullanılmıştır ve bu gruplar 35, 42, 65, 75, 100, 115 ve 125 mesh büyüklüğündeki parçalardan oluşmaktadır. Bu parçacıkların, ASTM standartlarına göre kül, nem ve lignin analizleri yapılmıştır. Analizi yapılan biyokütle, bilyalı değirmen ile öğütülmüştür. Bilyalı değirmende 35 mesh büyüklüğündeki lignoselülozik biyokütlenin, değişik işlem zamanlarında hangi boyuttaki parçacıklara küçültüldüğü ölçülmüştür. Öğütülen örnekler enzimle hidrolize edilmiş ve hidrolize edilen örneklerin selülaz enzim aktivitesi ölçülmüştür. Selülaz aktivitesinin ölçümü, enzimatik hidrolize uğrayan lignoselülozik biyokütleden alınan örneğin dinitrosalisiklik asit varlığında içerdiği toplam şeker miktarının ölçülmesidir. Bilyalı değirmen önişleminden sonra selülaz aktivitesi sonuçlarına göre seçilen büyüklükteki parçacıklara buharda patlatma işlemi uygulanmıştır. Buharda patlatma işleminde sıcaklık, azot basıncı, lignoselülozik biyokütle miktarı ve gaz basıncının boşaltım süresi değişken parametrelerdir. Yapılan deneylerde her seferinde bir parametre farklı tutularak şeker eldesine etkisi gözlemlenmiştir. Önişlemlerden sonra tüm örnekler Yüksek Performanslı Sıvı Kromatografisinde (HPLC) şeker analizleri yapılmıştır ve içerdikleri glikoz, galaktoz, arabinoz ve mannoz miktarları ölçülmüştür.

Bu çalışmada denenen koşullar sonucunda bilyalı değirmende öğütülen parçacıkların, selülaz aktivitesi ölçüldükten sonra 100, 115 ve 125 mesh büyüklüğünde en yüksek verimi gösterdiği görülmektedir fakat bu parçacıkların elde edilmesi zor olduğu için 75 mesh büyüklüğündeki parçacıklar ile buharda patlatma işleminin yapılmasına karar verilmiştir. 75 mesh büyüklüğündeki parçacıklar kısa sürede yüksek verimde elde edilmektedir. Ayrıca 75 mesh büyüklüğündeki parçacıklarda sadece bilyalı değirmen kullanımını şeker eldesinde çok yüksek verim edilmemektedir ve ek bir önişleme ihtiyaç duyulmaktadır. Bu nedenle buharda patlatma işlemi için uygun görülmüştür. Bu parçacıklar buharda patlatma işleminde 190°C'de, 64 psig azot basıncında buharda 10 dk bekletilerek ve ani basınç düşüşüyle patlatılarak en yüksek şeker eldesine sahip olduğu görülmüştür. Buna ek olarak, buharda patlatma işlemi sonucunda elde edilen katı biyokütlede glukoz ve mannoz oranı fazla iken elde edilen sıvı kısımda galaktoz ve arabinoz oranı fazladır. Bu da demek oluyor ki buharda patlatma işlemi sonucunda lignoselülozik biyokütle içerisindeki hemiselüloz, sıvı kısımda daha çok elde edilmektedir.

INTRODUCTION

Energy is one of the most significant necessities for humanity. Since human being, energy has been using in different types. In the world, the most common used energy source is fossil fuel. Fossil fuels are nonrenewable energy resources and they have finite reserves. Because of the increase in world population and industrial developments, energy demand has increased all around the world, fossil fuel reserves depleted and prices of fossil fuels are increased and this resulted as a research on new energy resources. At this point world is focusing on renewable energy sources.

Renewable energy sources include wind, solar, water power and biomass, all of which occur naturally on our planet. Biomass is an environmentally strategic energy resource, which can be produced with minimal requirements, and it also has significant influences on socio-economic development. Biomass energy technologies capture the energy stored in biomass and make it available in useful forms. Traditionally biomass has been utilized through direct combustion, and this process is still widely used in many parts of the world. Another means of utilizing it is realized through conversion technologies. From these technologies many solid, liquid, and gaseous biofuels can be obtained as alternative fuel candidates [nova]. Biofuels are a form of renewable energy produced from biomass and a clean alternative to fossil based fuels. Due to increasing world energy demand and high fossil based fuel prices, attention to biofuels are increasing. Using biofuels instead of fossil fuels has environmental and economic benefits and also it is significant to utilizing renewable sources and providing a variety of sources of energy. Biofuels are divided by 4 groups which are sorted by their feedstocks. These are 1^{st} generation biofuels which produced from agricultural products, 2^{nd} generation of biofuels which produced from lignocellulosic biomass and vegetable oil, 3^{rd} generation of biofuels which produced from genetically modified biomass and algae, 4^{th} generation of biofuels are known as carbon negative biofuels and produced from feedstocks with consummated genetics. Biofuels are bioethanol, biodiesel, biomethanol, biobuthanol, bioethyl tertiar buthyl ether, bio-methyl tertiar buthyl eter, bio-dimethylether,

biomethane, biohydrogen and biogas. Bioethanol is used as engine fuel, fuel of fuel cell, raw material for biodiesel production. Bioethanol is the most significant engine biofuel in the world and attention to bioethanol is increasing. Also in Turkey, bioethanol usage as an additive to gasoline is improving attention. The importance of bioethanol is produced from renewable biomass, decreased to dependency to fossil based fuels, decreased to emissions, increased to octane number.

Bioethanol is produced from starchy, sugary, lignocellulosic biomass. Bioethanol which is produced from starchy and sugary feedstocks is defined as first generation bioethanol. Bioethanol which is produced from lignocellulosic biomass, it is defined as second generation bioethanol. Lignocellulosic biomass, such as agricultural and forestry residues, waste paper, and industrial waste, mainly includes lignin, cellulose and hemicellulose are used for second generation biofuels production. For second generation ethanol production, lignocellulosic biomass is used as feedstock. Due to high lignin content of lignocellulosic feedstock, the pretreatment methods are needed to broke lignin structure and makes cellulose and hemicellulose part available for enzymatic hydrolysis. These pretreatment methods are physical pretreatment, chemical pretreatment, biological pretreatment, pulsed electric field pretreatment and physicochemical pretreatment. After pretreatment methods, pretreated lignocellulosic biomass hydrolyzed with enzyme. Products of enzymatic hydrolysis are 5 carbon – 6 carbon sugars such as glucose, galactose, xylose, mannose and arabinose. In fermentation step, these sugars are converted to bioethanol.

In this study, properties of lignocellulosic biomass, pretreatment technologies and production technologies are defined in theoretical section. The main aim of this study is measuring pretreatment effects on sugar content of lignocellulosic biomass. Pine wood is used as feedstock. Pine wood is pretreated according to ball mill and steam explosion pretreatment method and cellulase activity and sugar content are measured in experimental section.

THEORETICAL STUDY

Definition of biomass energy, biorefining technologies, classification of biofuels, properties of bioethanol, bioethanol pretreatment methods and production technologies are given in theoretical study section.

2.1 Biomass Energy

Biomass energy is a kind of renewable energy sources and obtained from biomass. Biomass is a general term for materials derived from growing plants and animal manure. Biomass energy resources include carbohydrate components and their origin come from plant and animal materials. Plant biomass is produced from photosynthesis by green plants and animal biomass is derived via plant biomass. There are many kinds of biomass energy sources. Some of biomass exists in water; some of them are on the land. It is a term used to describe all biologically produced matter. It refers to:

- Wood (energy forests and woody residues)
- Oilseed plants (sunflower, rapeseed, soybean, *etc.*)
- Carbohydrate plants (potato, wheat, corn, beet, *etc.*)
- Fiber plants (linseed, hemp plant, sorghum, *etc.*)
- Vegetal wastes (branch, stalk, straw, root, husk, *etc.*)
- Animal wastes
- Municipal wastes
- Industrial wastes
- Algae

Biomass energy sources are not homogeneous structure. It is meant that, the properties of all kinds of biomass are different from each other and also they include high ratio of water and oxygen, have low density and low heat value. Biomass energy is used as feedstock of biorefining technology [1].

2.2 Biorefining

Biorefineries which are used to produce biofuels such as oil refineries, but instead of oil they use biomass as feedstock. Currently a flexible product mixture that involves biochemicals, biomaterials, and biofuels, as well as the production of heat, cold, and electricity can be obtained by using different conversion technologies. In the 21st century the bioeconomy is widening and it is expected that biobased products and biofuels will be introduced into our daily life at an increasing rate [1].

Green Biorefinery is amulti product system which handles natural wet feedstocks derived from untreated products such as grass, green plants or green crops as input [2]. Industrial Biotechnology also known as "White Technology" is application to life sciences to traditional manufacturing and chemical synthesis. It uses microorganisms and enzymes like bacteria, fungi and special proteins to improve manufacturing processes. Industrial biotechnology makes biobased products and materials from renewable feedstocks. Industrial biotechnology makes agriculture more competitive and sustainable by creating non-food market, decrease environmental effects; help industries to provide sustainability and efficiency. It seen that Industrial Biotechnology has positive effect on society, environment and economy. In present day, the most commercialize cellulosic based ethanol production is white technology. Figure 2.1 shows the general concepts of biorefinery. Biorefinery doesn't concern to food and feed materials.

According to area of usage, biobased industrial products are divided 3 main groups. First group is biomaterials. Biomaterials include polymers, inks, paints, sheeting, adhesives, composite materials, construction materials. Second group is biochemicals and it includes surface absorber, by-product chemicals, lubrication oils and functional fluids. Third group is biofuels which are solid, liquid and gas biofuels [1].

Industrial biotechnology feedstocks are obtaining from biomass and also they are reliable, low cost, found in local areas and efficiency. Also, using biomass as a feedstock creates lower exhaust gases. The companies which use biotechnology will be able to generate marketable CO_2 credits or create internal regulatory flexibility. In this study, biofuels are investigated as a product of biorefinery.

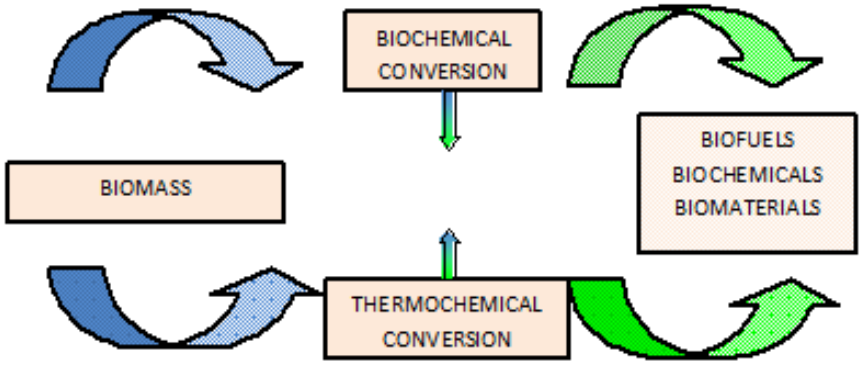

Figure 2.1 : Biorefining technology [1].

2.3 Biofuels

Biofuels are solid, liquid and gas fuel which are produced with various methods from different type of biomass and their properties are commercialize.

Biofuels are known as first generation biofuels and advanced generation biofuels. First generation biofuels are produced by present technology and their feedstocks are also food. Advanced generation biofuels are produced from non-food materials. Advanced generation biofuels include second, third and fourth generation biofuels.

First generation biofuels are produced from present technology and can be used internal combustion engines without any modification. Biodiesel, bioethanol, bioethyl tertiary buthyl ether and biogas are in this group. Agricultural products which are important input at food sector are used to product biodiesel and bioethanol, wastes are used to product biogas [3].

Vegetable oil, biodiesel which is produced from oil acid ethyl ester, bioethanol which is produced from lignocellulosic feedstocks, biomethanol, biobuthanol, bioethyl tertiary buthy ether, biomethyl tertiary buthy ether, biodimethyl ether, biomethane and biohydrogen which are produced from biomass conversion technologies and liquid fuel technology products as Fischer-Tropsch Diesel and Fischer-Tropsch Gasoline are in the group of second generation biofuels. All this fuels are produced from lignocellulosic feedstocks and feedstocks are not food. The production and usage of second generation biofuels are high cost. In the present day, the cost of second generation biofuels are greater than petroleum fuels and traditional biofuels and also technological sufficiency does not obtain. New technological developments require for fermentation, pretreatments and enzymes to decrease cost.

To be commercially, new ground works need for transportation, storage and refining [3].

Third generation biofuels consist of genetically modified plants and algaes which are including high oil and cellulose. These types of biofuels are used at integrated biorefining technologies. Feedstocks of biofuels will turn to cellulosic sources instead of lignocellulosic sources. Third generation biofuels are also known as ‘‘Advanced Biofuels’’ and the aims of advanced biofuels are storage of carbon dioxide and being high efficient.

Fourth generation biofuels are known as ‘‘Carbon Negative Biofuels’’ and will be produced from consummated genetics feedstocks. With developed technologies like sequestration and carbon holding, lower emissions of carbon dioxide (CO_2) will be released to the environment [1].

Widespread use of biofuels may increase national security as domestic biomass is substituted for foreign oil in fuel production [4].

Next section is included bioethanol properties, feedstocks, pretreatment technologies and production.

2.4 Bioethanol

The history of bioethanol, which is today the world’s leading engine biofuel, begins with the history of internal combustion engines. N. A. Otto used bioethanol in studies with engines and Henry Ford took the combustion of alcohols into account. Scientific studies were intensified, especially during the Second World War, and research and application studies increased after the oil crisis of 1974 [1].

Ethanol (EtOH), also known as ‘‘Ethyl alcohol’’, ‘‘Grade alcohol’’ and “Fuel alcohol”. It’s molecular formula C_2H_5OH [5]. Bioethanol is also named depend on its feedstock as an example, cellulosic bioethanol is produced from cellulosic biomass; lignocellulosic bioethanol is produced from lignocellulosic biomass. Bioethanol had been used successfully as an engine fuel in 1908 by Henry Ford. Bioethanol is produced by microbial conversion from biomass through fermentation [1]. Table 2.1 shows that physical, chemical and thermal properties of bioethanol.

Table 2.1 : Properties of bioethanol [5].

Properties	**Values**
Formula	C_2H_5OH
Molecular Weight (g/mol)	46.1
Carbon (w/w, %)	52.1
Hydrogen (w/w, %)	13.1
Oxygen (w/w, %)	34.7
C/H ratio (wt)	4
Specific Weight (kg/L)	0.79
Vapor Pressure (at 38 ^oC) (mmHg)	50
Boiling Temperature (^oC)	78.5
Solubility in Water	∞
Stoichiometric (air/EtOH)	9
Lower Heating Value (kcal/kg)	6400
Ignition Temperature (^oC)	35
Specific Heat (kcal/kg ^oC)	0.6
Melting Point (^oC)	-115

Because of having high octane number, low cetane number and high heat of vaporization, bioethanol has been recognized as a potential alternative to petroleum derived transportation fuel [6]. Bioethanol should be used in gasoline engines. However, ethanol doesn't use directly only fuel in engines without modification, it uses as additive. Bioethanol can be used as alternative fuel, fuel additive, fuel for fuel cells and feedstock for production of biodiesel and bioethyl tertiary buthly ether. In present, there are lots of vehicles which use 100% bioethanol however, using bioethanol as an additive for gasoline is legal obligation in some countries such as Brazil. In this project, the ethanol which is used for fuel is examined.

Gasoline with additive alcohol: the gasoline which includes maximum 5% alcohol

Gasohol (E10) : the fuel which includes 10% alcohol and 90% gasoline

E20 : the fuel which includes 20% alcohol and 80% gasoline

E25 : the fuel which includes 25% alcohol and 75% gasoline

E85 : the fuel which includes 85% alcohol and 15% gasoline

E-diesel (Oxydiesel) : the diesel which includes maximum 15% alcohol

Gasohol is the primary alternative for gasoline because of its high performance and clean burning characteristics, and Oxydiesel is the alternative for diesel fuel [1].

2.5 Feedstocks for Bioethanol Production

Sugar crops, starch crops and lignocellulosic materials are used for bioethanol production. Today, the most common used feedstocks for production bioethanol are raw sugars from sugarcane or sugar beet, or starch found in the grain cereal crops. Bioethanol which is produced from these types of materials is first generation bioethanol [4].

In addition to providing the source for the manufacture of sugar, sugar crops are used to produce alcohol and ethanol. In certain countries, sugar cane is eaten raw in minor quantities. It also is used in the preparation of juices and for animal feed. World sugar production from sugarcane is two-thirds of the world and from sugar beet is one-third. Sugar cane is grown in tropical and subtropical countries, while sugar beet is grown only in temperate climate countries. Both sugar beets and sugar cane have a high water content, accounting for about 75 percent of the total weight of the plants. The sugar content of sugar cane ranges from 10 to 15 percent of the total weight, while that of sugar beets is between 13 and 18 percent. The protein and fat content of both beets and cane is almost zero and it makes sugar beet and sugar cane are very important feedstock for bioethanol production [6,7].

There are a high number of starch sources, however only a few of them have industrial importance. Most important starch sources are corn, sorghum, barley, oats, maize, cassava, potato and wheat [4,6]. Corn is a well-suited feedstock for bioethanol production because of its high starch content. Starch content of corn kernel is 72% (w/w). Average production yield of corn is 9.6 t/ha and 3.1 t/ha for wheat in 2010. For sorghum, barley and oats, average production yield is beyween 1.3 to 2.3 t/ha [4]. Table 2.2 shows that, bioethanol conversion for sugar and starch crops

Table 2.2 : Bioethanol conversion efficiency for different feedstocks [8].

Feedstock	Tone/hectare	Sugar (%)	Ethanol (L/tone)	Production (L/hectare)
Sugar Cane	50-100	13	60-80	3500-7000
Sugar Beet	40-50	16	90-100	3800-4800
Corn	4-8	60	360-400	1500-3000
Wheat	2-9	62	370-420	740-3800
Sorghum	4-15	70	330-370	1480-6300
Harep	8-10	45	150	1380

As it is stated to Table 2.2, bioethanol conversion efficiency is highest than other materials. 3500 – 7000 L bioethanol is produced from 1 hectare sugar cane planted area. Efficiency of sugar beet and sorghum are quite high. 3800 - 4800 L bioethanol is produced from 1 hectare sugar beet planted area and 1480 – 6300 L bioethanol is produced from sorghum. The most common used bioethanol feedstock corn converts 1500 – 3000 L bioethanol.

The use of sugar and starch as feedstock for fuel production competes with their use as foods. Because of that, lignocellulosic biomass is an effective alternative feedstock for fuel ethanol production and lignocellulosic bioethanol is second generation bioethanol [6].

Lignocellulose is the botanical term and use for woody or fibrous plant materials so lignocellulose is the most obtainable renewable resource on Earth. Lignocellulosic biomass constitutes a large component of the wastes originating from municipal, agricultural, forestry and industrial sources. Using lignocellulosic biomass provides security of supply more than fossil reserves because of distributing widespread geographical area and lignocellulosic materials are cheap and available feedstocks that the industry will be able to access [4,6].

Lignocellulosic materials combined with lignin, cellulose and hemicellulose. In Figure 2.2, chemical structure of lignocellulosic biomass is shown.

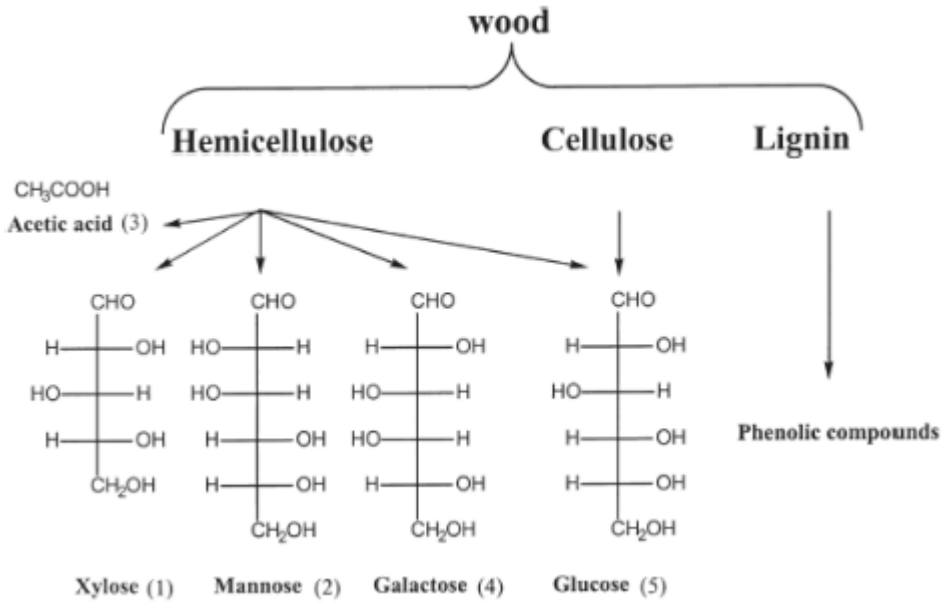

Figure 2.2 : Chemical structure of lignocellulosic biomass [6].

Cellulose molecules consist of long chains of glucose molecules connected via (1,4)-β-D linkages [4]. However their structural configuration is different for starch molecules. Hemicellulose is also comprised of long chains of sugar molecules; but contains, in addition to glucose (a 6-carbon or hexose sugar), contains pentoses (5-carbon sugars), the main function of lignin is to provide structural support for the plants. Hemicellulose is a polysaccharides and its molecular weight is lower than cellulose [6]. It is formed from D-xylose, D-mannose, D-galactose, D-glucose and L-arabinose which are seen in Figure 2.2. Lignin is composed of a number of phenolic compounds that may act as an inhibitor. Having high rate of lignin make to reach cellulose and hemicellulose is difficult and complicated. In Figure 2.3 and Figure 2.4 are shown that, structure of cellulose, glucose, galactose, mannose, arabinose and xylose.

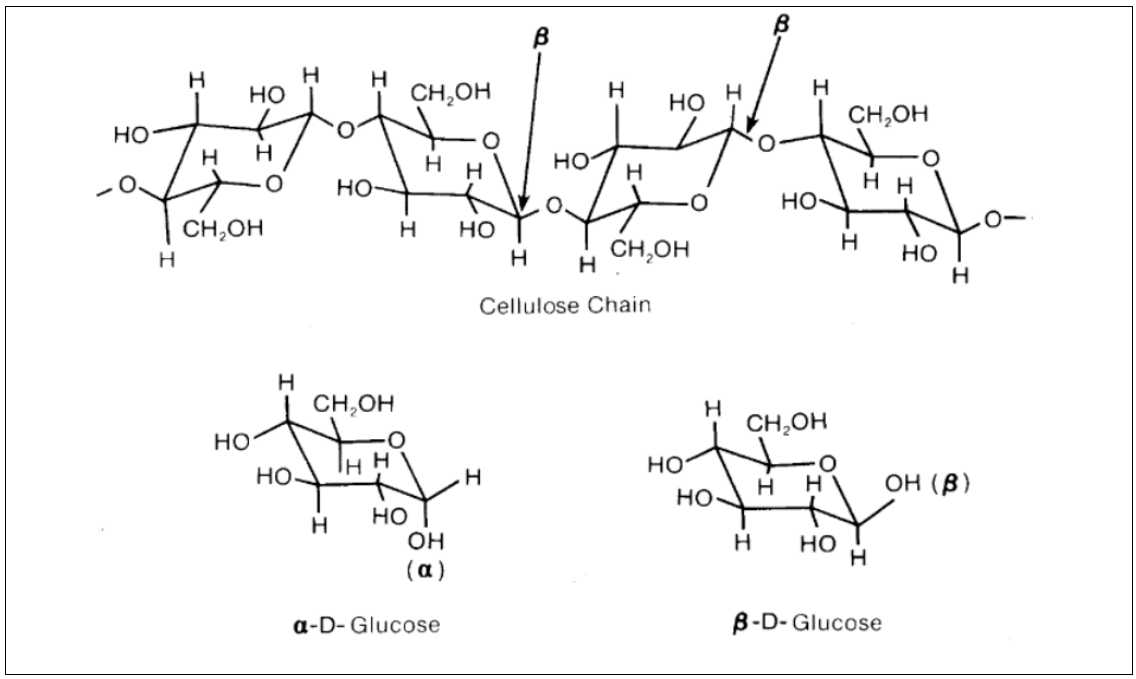

Figure 2.3 : Chemical structure of cellulose chain and α and β –D-glucose.

Implementing a bioconversion system utilizing lignocellulosic feedstocks requires some separation methods of cellulose and hemicellulose from lignin. Because of this, lignocellulosic based bioconversion is more difficult and expensive. However; lignocellulosic materials does not affect food chain as sugar and starch crops and thus an important aspect of food security is preserved [4]. Bioethanol from lignocellulosic biomass is second generation biofuel. Table 2.3 shows that lignin, cellulose and hemicelluloses content of some feedstocks [9].

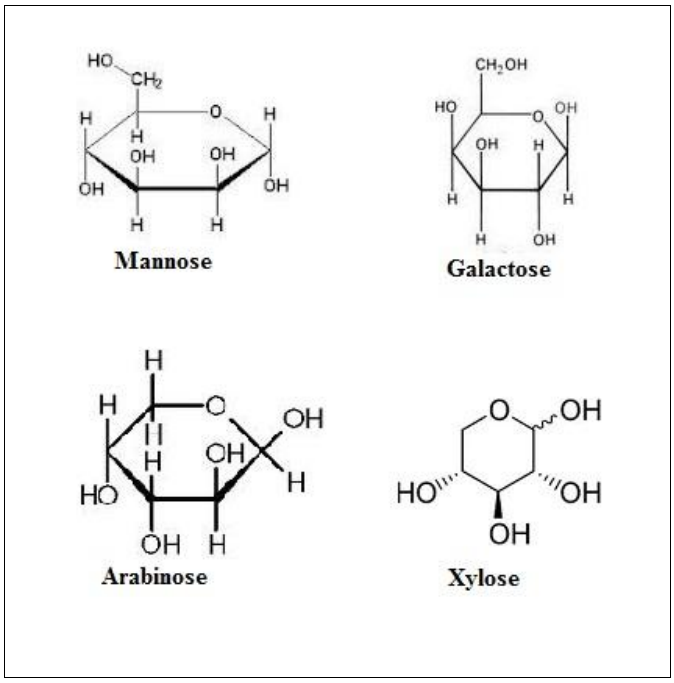

Figure 2.4 : Chemical structure of mannose, galactose, arabinose and xylose.

Table 2.3 : Cellulose, hemicellulose and lignin content of materials (%, w/w) [9].

Lignocellulosic Materials	Cellulose	Hemicellulose	Lignin
Hardwood stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA	24–29
Swine waste	6.0	28	NA
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

As it seen in Table 2.3 that, cellulose content of hardwood and softwood stems are higher than hemicellulose and lignin content. Lignin content of nut shells is higher than cellulose and hemicellulose. Corn crabs have high cellulose content.

Hemicellulose and cellulose content of grasses is more than lignin content. In wheat straw, cellulose content is higher than lignin content; however lower than hemicellulose content. Cellulose content of sorted refuse is higher than both hemicellulose and lignin content. Leaves have high ratio of hemicellulose content and contains no lignin. Huge amount of cellulose are included cotton seed hairs. Hemicellulose and lignin content of newspaper are approximate values. Wastes content are not defined absolutely because of their content is variable. Solid cattle manure contains very small amount of cellulose, hemicellulose and lignin. Coastal Bermuda grass contains high hemicellulose and switch grass contains high cellulose. In general, the biomass of hardwood species has more cellulose than softwoods; however, has less hemicellulose than softwoods. It is meant that, after hydrolysis step, greater amounts of glucose are available in hardwoods [4]. According to Table 2.3, paper contains most cellulose than other materials which shown in table. In Figure 2.5, structure of plant cell is shown.

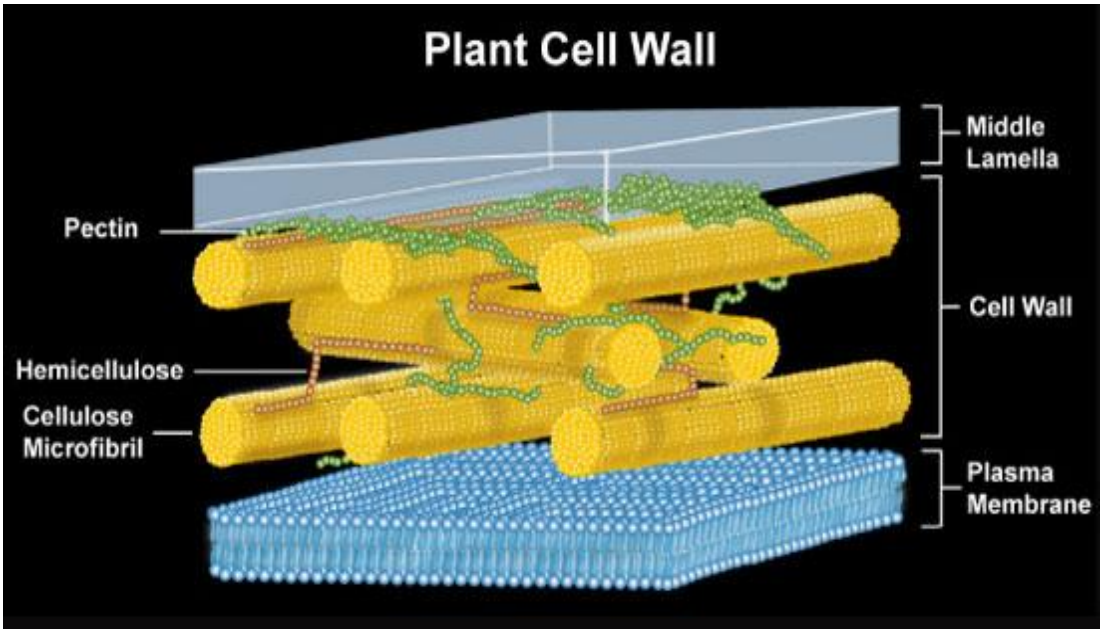

Figure 2.2 : Structure of plant cell.

As it is seen in Figure 2.5 that, plant cell is consist of middle lamella, cell wall and plasma membrane. Middle lamella is the first layer formed during cell division. It makes up the outer wall of the cell and is shared by adjacent cells. It is composed of pectic compounds and protein [10]. Cell wall includes hemicellulose and cellulose microfibril. The plasma membrane (cell membrane) is made of two layers of phospholipids. The membrane has many proteins embedded in it. The plasma

membrane regulates what enters and leaves the cell. Many molecules cross the cell membrane by diffusion and osmosis [11].

2.6 Bioethanol Production Technologies

Bioethanol is produced by two methods. These methods are thermochemical method and bioconversion method. Steps of thermochemical method are pretreatment, gasification, cleanup, generating synthesis gases which include hydrogen and carbon monoxide and producing Fischer-Tropsch fuel such as methanol and ethanol [4]. Bioconversion method for lignocellulosic bioethanol production is described below.

In Figure 2.6, lignocellulosic bioethanol production via bioconversion method is shown. As it is seen in Figure 2.6 that, lignocellulosic biomass is used as feedstock. Then, lignocellulosic biomass is prepared to hydrolysis with pretreatment methods. Pretreated samples are hydrolyzed with enzyme and acid. Sugar solution is become after hydrolysis. Sugar solution is fermentated and it is converted to bioethanol solution. Bioethanol solution is distilled to increase bioethanol density. After distillation ethanol is 90-95 % (w/w) and after dehydration, bioethanol is produced.

2.7 Pretreatments of Lignocellulosic Feedstock

Pretreatment of lignocellulosic biomass for bioethanol production is important because the main challenge in second generation bioethanol production from lignocellulosic biomass is converting complex polymers into simple sugars and decreasing negative effect of lignin content of lignocellulosic biomass. Therefore, pretreatment methods are applied [6]. The aim of pretreatment is to remove lignin and hemicellulose from cellulose and to make cellulose more accessible for hydrolysis [12]. On the other hand, pretreatment processes reduce crystallinity of cellulose and increase the porosity of the lignocellulosic material. Pretreatment processes must increase the formation of sugars or increase the ability of subsequently form sugars which produced from hydrolysis, prevent the degradation or loss of carbohydrate, avoid the formation of byproducts which are decrease the yield of hydrolysis and fermentation process and must be cost effective [12]. Figure 2.7 shows that the structure of plant cell after pretreatments.

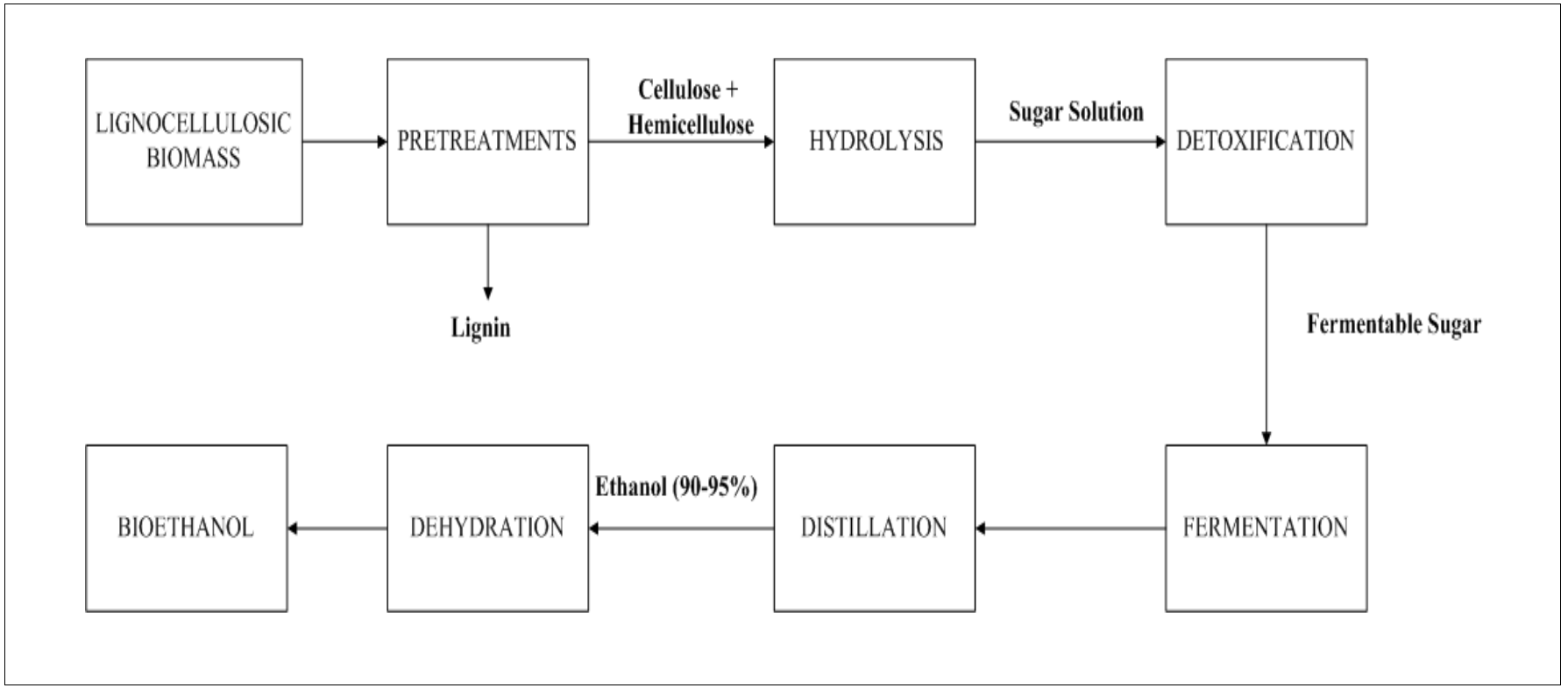

Figure 2.6 : Lignocellulosic bioethanol production via bioconversion method.

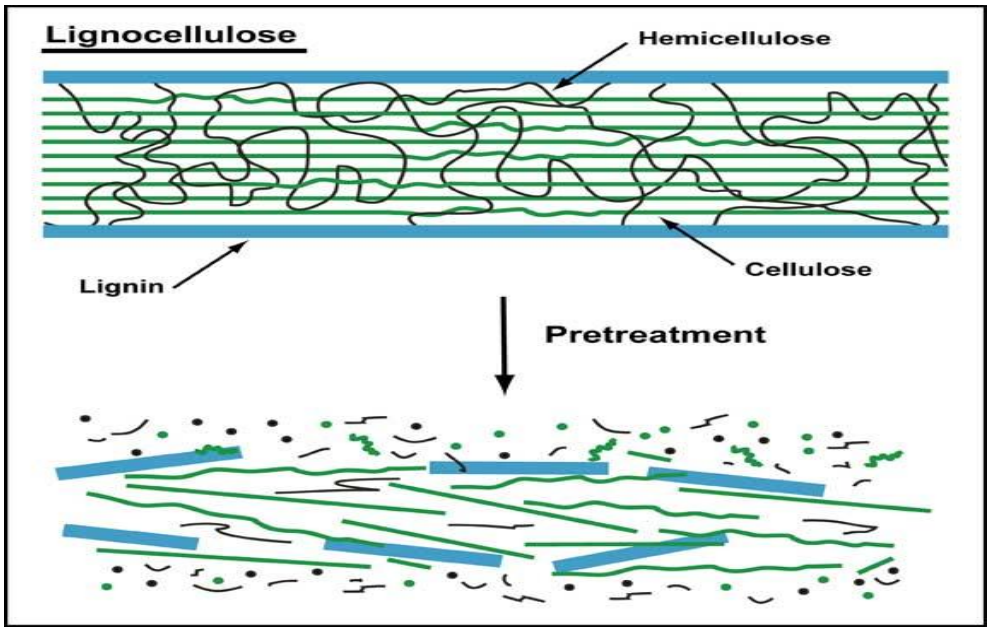

Figure 2.7 : Structure of plant cell after pretreatment.

As it is seen in Figure 2.7 that, after pretreatment, the lignin part of cell is broken and enzymes can easily reach cellulose and hemicellulose part of cells. 33% of total cost of bioethanol production is being pretreatment. The chemistry of the pretreatment is important due to impact on the global ethanol production process and also the cost of the operational steps which are determining fermentation toxicity, enzymatic hydrolysis rates and enzyme loading are affected by pretreatment methods. Sugar recovery yield, chip size required and low energy demand have been described as important parameters for effective pretreatment methods [6]. Key factors of pretreatment which reduce cost and advance pretreatment technologies are detailed below;

- High yields for multiple crops, sites ages, harvesting times; various pretreatments have been shown to be better suited for specific feedstocks. For example, alkaline-based pretreatment methods such as lime, ammonia fiber explosion (AFEX), and ammonia recycling percolation (ARP), can effectively reduce the lignin content of agricultural residues but are less satisfactory for processing recalcitrant substrate as softwoods. Acid based pretreatment processes have been shown to be effective on a wide range of lignocelluloses substrate, but are relatively expensive.

- Highly digestible pretreated solid; cellulose from pretreatment are preferred to be highly digestible with yields higher than 90% in less than five or less than 3 days with enzyme loading lower than 10 FPU/g of cellulose.
- Significant sugars should not be degradation; high yields close to 100% of fermentable cellulosic and hemicellulosic sugars should be achieved through pretreatment step.
- Minimum amount of toxic compounds; the liquid hydrolyzate from pretreatment must be fermentable following a low-cost, high yield conditioning step. Harsh conditions during pretreatment lead to a partial hemicellulose degradation and generation of toxic compounds derived from sugar decomposition that could affect the proceeding hydrolysis and fermentation steps.
- Toxic compounds generated and their amounts depend on feedstock and harshness of pretreatment. Degradation products from pretreatment of lignocelluloses materials can be divided into the following classes: carboxylic acids, furan derivatives, and phenolic compounds. Main furan derivates are furfural and 5-hydroxymethylfurfural (HMF) derived from pentoses and hexoses degradation, respectively; Weak acids are mostly acetic and formic and levulinic acids phenolic compounds include alcohols, aldehydes, ketones and acids.
- Biomass size reduction should be minimized. Milling or grinding the feedstock to small particle sizes before pretreatment is energy-intensive and costly technologies.
- Operation in reasonable size and moderate cost of reactors; pretreatment reactors should be low in cost through minimizing their volume, employing appropriate materials of construction for highly corrosive chemical environments, and keeping operating pressures and temperature reasonable.
- Non-production of solid-waste residues; the chemicals formed during hydrolyzate conditioning in preparation for subsequent steps should not present processing or disposal challenges.
- Effectiveness at low moisture content; for reducing energy consumption during pretreatment, feedstocks should have high dry matter content.
- Obtaining high sugar concentration; the concentration of sugars from the coupled operation of pretreatment and enzymatic hydrolysis should be above 10%.

- Fermentation compatibility; the distribution of sugar recovery between pretreatment and subsequent enzymatic hydrolysis should be compatible with the choice of an organism able to ferment pentoses (arabinose and xylose) in hemicellulose.
- Lignin recovery; Lignin and other constituents should be recovered for conversion into valuable co-products.
- Minimum heat and power requirements; Pretreatment's heat and power demands should be lower than the thermally integrated process for being competitive [13].

Pretreatment Technologies can be divided several groups. Pretreatment methods are shown in Figure 2.8. According to Figure 2.8, there are 5 types of pretreatment methods are exist. These are physical pretreatment, chemical pretreatment, biological pretreatment, pulsed electric field pretreatment and physicochemical pretreatment. Pretreatment methods are detailed in next section.

2.7.1 Physical pretreatment

Physical pretreatments are usually first step of pretreatment methods and main aim of physical pretreatment is reducing size of lignocellulosic biomass and breaking the physical structure [6]. Mechanical comminution and extrusion are included physical pretreatment. Physical pretreatments affect only physical properties of lignocellulosic material.

Mechanical comminution is the most commonly used and most effective physical pretreatment method for lignocellulosic materials. The aim of mechanical comminution is decrease to size of feedstock which causes to increase surface area of feedstocks, reduce the degree of polymerization and enhancing enzyme accessibility to cellulose, breaking the physical structure and reduce the degree of polymerization. Although using very fine particle as a feedstock is increased to bioethanol yield, it is also increased energy consumption so it is not preferred [6]. Mechanical comminution includes chipping, grinding and milling. The material size after chipping is usually 10 to 30 mm and after milling and grilling is 0.2 to 2 mm [6,12]. Balls, blades shoul be used for mechanical comminution.

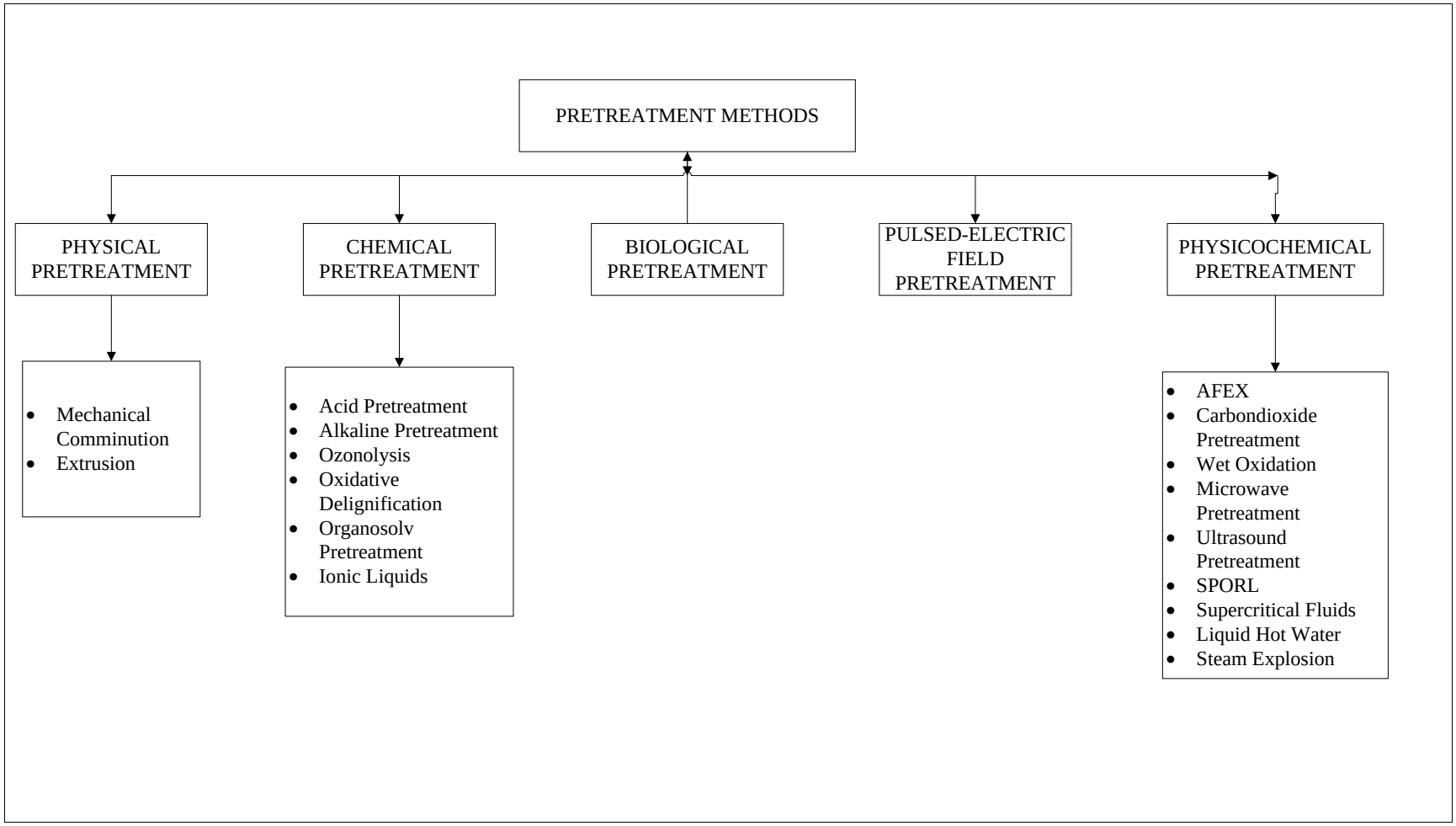

Figure 2.8 : Pretreatment methods for lignocellulosic biomass.

Extrusion is a promising physical pretreatment method for lignocellulosic biomass conversion to bioethanol. In this pretreatment method, lignocellulosic materials are exposed to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder. Ability to provide high shear, rapid heat transfer and effective and rapid mixing are advantages of extruder. The lignocellulosic structure causing defibrillation and shortening of the fibers are disrupted due to screw speed and barrel temperature. Therefore, it is increasing accessibility of carbohydrates to enzymatic attack. Extrusion has high adaptability to many different process modifications such as addition of chemicals or removal of materials and the application of high pressure and expansion treatment which is using steam or other solvent. Due to these properties, extrusion becomes a novel pretreatment method for lignocellulosic biomass [6].

2.7.2 Chemical pretreatment

In acid pretreatment, acids are used as catalyst which has stronger effect on hemicellulose and lignin than cellulose. Acid pretreatment methods are used for breaking linkages in hemicellulose and cellulose and making cellulose more accessible to enzymes. In acid pretreatment method, different types of reactors are used such as percolation, plug flow, shrinking-bed, batch and counter-current reactors. Two kind of acid pretreatment methods are applied which are concentrated acid pretreatment and dilute acid pretreatment. Sulfuric acid is used in both concentrated and dilute acid pretreatment more than hydrochloric acid, nitric and trifluoracetic acids (TFA). Concentrated acids are effective hydrolysis agents and this method are used for hydrolysis both cellulose and hemicellulose. In concentrated acid pretreatment method, H_2SO_4 and HCl are most common used acids. The advantage of concentrated acid pretreatment is operation conditions are moderate. Operating at low/medium temperatures and requiring no enzyme decrease operational cost, however equipment corrosion problems and acid recovery are important problems in concentrated acid pretreatment. Therefore, dilute acid pretreatment is more applicable for industrial scale plants. Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic biomass. Both hemicellulose and cellulose are affected by dilute acid pretreatment. Dilute acid pretreatment yield sugar recoveries from hemicellulose are between 70 – 95%. In dilute acid pretreatment, phosphoric acid and weak organic acid are used which are

0.5 – 1.5% concentration. Dilute acid hydrolysis has reached high reaction rates and cellulose hydrolysis has been improved significantly. Dilute acid hydrolysis at high temperature is appropriate for cellulose hydrolysis. In generally, two types of dilute acid pretreatment are used which are high temperature (T > 160°C), continuous-flow process for low solid loading (5 - 10%, weight of substrate/weight of reaction mixture) during short period time and low temperature (T < 160°C), batch process for high solid loadings (10 - 40%) for longer retention time (30 - 90 min). Some sugar degradation compounds such as furfural, HMF and aromatic lignin degradation compound are detected depending on process temperature and these undesirable compounds affect microorganism metabolism in the fermentation step. These undesirable compounds are generates by dilute acid pretreatment lower than concentrated acid pretreatment. Both dilute and concentrated acid pretreatment, the acid has to be removed and neutralized before fermentation [6,9,12-15].

Alkali based pretreatment such as sodium hydroxide pretreatments, lime pretreatment, ammonia-based pretreatments and ionic liquid pretreatment effects of the pretreatment depend on the lignin content of materials. Alkali based pretreatment requires lower temperature and pressure than other pretreatment methods. Both alkali based pretreatment can improve the cellulose digestibility and sugar degradation is lower than acid pretreatments. NaOH, KOH, $Ca(OH)_2$ and NH_4OH are used for alkali pretreatment. NaOH causes swelling so surface area of cellulose is increasing and the degree of polymerization and cristallinity are decreasing. Hardwood digestibility is increased from 14% to 55% and lignin content is reduced from 24-55% to 20%. Other alkali, $Ca(OH)_2$ is also known as lime, removes acetyl groups from hemicellulose and increases cellulose digestibility. Alkali pretreatment with lime is operating successfully at temperature between 85°C and 150°C for 3-13 hours. For separating solid fraction to alkali, pH is decreased; however this separation step is not interesting owing to the great amount of fermentable sugars present in the liquid fraction. Lime pretreatment has lower cost and more safety than NaOH and KOH pretreatments. Lignin removing performance is increased to alkaline pretreatment with oxidant agent such as oxygen and H_2O_2. Moreover, no furfural and HMF are detected after alkaline pretreatment. [6,12,16-17].

Ozone is an oxidizing agent that shows high delignification efficiency. Usually, ozonolysis is operating at room temperatures and atmospheric pressure. Advantages

of ozonolysis pretreatment are it removes lignin effectively, doesn't produce toxic residues for the downstream processes, it is operating at atmospheric conditions. Ozone has been used to pretreat several lignocellulosic biomass such as wheat straw, rye straw, cotton straw, bagasse and poplar among others. Enzymatic hydrolysis yield is increased from 0% to 57% as the percentage of lignin decreased from 29% to 8%. Despite of these results, ozonolysis pretreatment is not applied to lignocellulosic biomass due to requiring large amount of ozone [6,9,12,18,19].

In oxidative delignification pretreatment, lignin biodegradation could be catalyzed by the peroxidase enzymes with the presence of H_2O_2. About 50% of lignin and high amount of hemicellulose were solubilized by 2% H_2O_2 at 30°C with 8 hours and 95% efficiency of glucose production from cellulose was achieved in the saccharification step by cellulose at 45°C for 24 hours [9, 20].

In organosolv pretreatment method, organic and aqueous solvents such as ethanol, methanol, ethylene glycol, acetone, glycerol and tetrahydrofurfuryl alcohol are used to exact lignin and provide more accessible cellulose. The organic solvent is mixed with different portions of water and added to biomass. This mixture is heated to temperature ranging between 100 - 250°C. If the process is operated at low temperature as 180 - 210°C, some acids such as HCl, H_2SO_4, oxalic and salicyclic can be added as catalyst to break hemicellulose bonds. Cellulosic fibers, solid lignin which is obtained from volatile solvent after removal and liquid solution of hemicellulose are obtained from lignocellulosic biomass after organosolv process. Due to inhibiting to enzymatic hydrolysis and fermentative microorganisms, removing solvents from process is necessary using appropriate extraction and separation techniques such as evaporation and condensation. The main advantage of organosolv pretreatment than other chemical pretreatments is recovering a relatively pure lignin as a byproduct. On the other hand, high commercial solvent prices increase the importance of recycling of solvents and also recycling is decreasing operational cost. Depending on economical reasons, low molecular weight alcohol with lower boiling such as ethanol and methanol are most common used solvents in organosolv pretreatment because of obtained easily by distillation. Significant amount of furfural, HMF and soluble phenols from lignin are obtained after organosolv pretreatment. Organosolv pretreatment has been suggested to use with

acid hydrolyss to separate hemicellulose and lignin in a two-stage fractionation [6,9,14,21-25].

During the last decade, the use of ionic liquids (ILs) as solvents for pretreatment of cellulosic biomass has more attention. ILs are salts with melting points usually below 100°C, typically composed of large organic cations and small inorganic anions which exist as liquids at relatively low temperatures. They are break down the extensive hydrogen bonding network in the polysaccharides and promote its solubilization. The significant characteristics of ILs are their thermal and chemical stability, nonflammability, wide liquid temperature range and good solvating properties for various types of materials. Some ILs are defined as "green" solvent which has not formed non toxic and explosive gases. ILs pretreatment can be applied to lignocellulosic biomass such as bagasse, wheat straw and wood. Solvents which applied to lignocellulosic biomass for ILs pretreatment are 1-ethyl-3-methylimidazolium acetate and 1-allyl-3-metilimidazolium chloride. Using ILs is the limited amount of data about their toxicity and biodegradability [14, 26-32].

2.7.3 Biological pretreatment

In biological pretreatment processes, microorganism such as brown, white and soft fungi are used to degrade lignin and hemicellulose in structure of lignocellulosic biomass. White rot fungi with selectivity to lignin degradation over cellulose can be successfully applied in microbial pretreatments. White fungi have the highest efficiency in biologic pretreatment microbials. Brown rots mainly attack cellulose, although white and soft rots attackboth cellulose and lignin.

Opposite to other pretreatment technologies such as dilute acid, steam explosion, hydrothermal and alkali extraction, biological pretreatment method of lignocellulosic biomass is defined as environmental process due to no use of chemicals, reducing energy input, no require pressured and corrosion-resistant reactors, no waste stream generated and minimal inhibitor production. These properties are advantages of biological pretreatment method. However, obtaining low hydrolysis rate and requiring longer retention time than other pretreatment methods are disadvantages of biological pretreatment method [9, 33-37].

2.7.4 Pulsed – electric – field pretreatment

Pulsed - electric - field (PEF) pretreatment includes a short burst of high voltage to a ample between two parallel plate electrodes. The field strength is equal to voltage per distance for two electrodes. PEF causes dramatic increase in mass permeability and sometimes mechanical rupture of plant tissue and also create permanent pores in the cell membrane and hence facilitate the entry of acids or enzymes ysed to break down the cellulose into its constituent sugars. Pulses are applied in the form of exponential decay or square waves. The duration of PEF pretreatment is between nanoseconds to microseconds. In PEC pretreatment methods electric field strength, the number of pulses and treatment time are significant variables. Generally, pulse generator, treatment chamber, data acquisition - control system and material – handling equipment are required for PEF pretreatment. Main advantages of PEF pretreatment methods are requirement low energy due to short retention time and equipments are not complex because of having non-moving particles [12].

2.7.5 Physicochemical pretreatment

Physicochemical pretreatment methods separate to component of lignocellulosic biomass such as lignin, hemicellulose and cellulose, reduce the crystalline structure of cellulose [6]. Physicochemical pretreatment includes ammonia fiber explosion, carbon dioxide explosion, wet oxidation, microwave and ultrasound pretreatment, sulfite pretreatment to overcome recalcitrance of lignocellulose, liquid hot water and steam explosion.

One of the physicochemical pretreatment methods is ammonia fiber explosion method (AFEX). In the AFEX pretreatment method, biomass is treated with anhydrous ammonia in liquid form at temperatures between 60°C and 100°C and high pressure for a variable period of time. After the retention time, the pressure is released suddenly, vaporizing the ammonia and allowing its recovery and recycling. Generally, the dosage of liquid ammonia is 1-2 kg of ammonia/kg of dry biomass, the temperature is 90°C and retention time is 30 minutes. Temperature is the most significant variable in the AFEX pretreatment method. Temperature determines the amount of ammonia vaporized during the explosive flash and influences the system pressure. At higher temperatures, more ammonia vapors flash and therefore greater disruption of the biomass fiber structure probably occurs. In optimal conditions,

AFEX can achieve more than 90% conversion of cellulose and hemicellulose to fermentable sugars. The ammonia has significant effect on lignocellulosic biomass for causing swelling and physical disruption of biomass fibers, partial decrystallization of cellulose and breakdown of lignin carbohydrates linkages. However, the AFEX process is not effective for lignocellulosic biomass which includes high content of lignin [6, 12, 38].

The supercritical fluids such as water, carbon dioxide, and ammonia are compounds that are in a gaseous form but are compressed at temperatures above their critical point to a liquid like density. Supercritical pretreatment conditions can remove lignin increasing substrate digestibility highly. Carbon dioxide explosion is the most common used supercritical fluids. CO_2 is nontoxic, noninflammable, leaves no harmful residues, and is inexpensive and readily available. In aqueous solution, CO_2 forms carbonic acid, which favors the polymers hydrolysis. In CO_2 pretreatment process, biomass is facilitated by high pressure. After the explosive release of CO_2 pressure, disruption of cellulose and hemicellulose structure is observed and consequently accessible surface area of the substrate to enzymatic attack increases. Operation at low temperatures compared to other methods prevents monosaccharides degradation, but in comparison to steam and ammonia explosion sugar yields obtained are lower. A comparison of different pretreatment methods on several substrates assest that CO_2 explosion was more cost effective than AFEX and formation of inhibitors was lower compared to steam explosion. Current efforts to develop these methods do not investigate economic fisibilty. A very high-pressure requirement is a concerning issue. On the other hand, CO_2 utilization could be an attractive alternative to reduce costs because of its coproduction during ethanol fermentation [6, 39-40].

Wet oxidation is an oxidative pretreatment method which employs catalyst such as oxygen or air. The main difference of wet oxidation pretreatment method from steam explosion is presence of oxygen. Wet oxidation pretreatment is operating at 170 - 200°C during 5 - 15 minutes at pressure from 10 – 12 bar O_2. Oxygen is added at temperatures above 170°C makes the process exothermic reducing the total energy demand. As it is stated in articles that, wet oxidation is an efficient method for solubilization of hemicellulose and lignin. However, wet oxidation does not catalyze the hydrolysis of solubilized hemicellulose. When steam explosion and dilute acid

pretreatment produce sugar monomers, wet oxidation pretreatment produces soluble sugars from hemicellulose as oligomers. However, furfural, and HMF production is lower than steam explosion or liquid hot water pretreatment. The formation of toxic is decreasing for adding carbonate (Na_3CO_2) in wet oxidation and it is called alkaline wet oxidation. After wet oxidation pretreatment, the yield of enzymatic hydrolysis has been high. This pretreatment are common used for bioethanol production from corn stover, clover-ryegrass and olive pulp. The main disadvantages of wet oxidation pretreatment are cost of oxygen and catalyst [13, 41-47].

Microwave based pretreatment combines both thermal and nonthermal effects generated in aqueous environment. Ions movement and polar molecules vibration increase heat and extensive intermolecular collision which accelerate chemical, physical and biological process. Microwave based pretreatment is operating in dilute chemical reagents and exposing the slurry to microwave radiation for retention time ranging from 5 to 20 minutes. Opposite to conduction or convection heating, microwave uses the ability of direct interaction between a heated object and an applied electromagnetic field to increase heat. Using microwave pretreatment instead of conventional heating decreases of process energy requirements and causes uniform and selective processing. Microwave pretreatment is faster because of heat is generated via direct interaction between the electromagnetic field and components of heating material. Electromagnetic field helps to accelerate the destruction of crystalline structure and changes super molecular structure of lignocellulosic biomass increases its reactivity with fermentative microorganisms. As a result, the short length of pretreatment and low inhibitor production are reflected in high cost efficiency. However, industrial scale application is unknown for microwave pretreatment [13, 48, 49].

Ultrasound which is known as the mechanical waves at frequency above the hearing range for humans, has applied biological and chemical processes. Ultrasound pretreatment is extracting to hemicellulose, cellulose and lignin from lignocellulosic biomass and also it increases the conversion of starch materials to glucose and therefore improves the ethanol yield. Just a few recent studies are defined hydrolysis performance to ultrasound pretreated lignocellulosic biomass. In general concept, ultrasound waves produce cavitations and acoustic streaming in a liquid or slurry. After ultrasound pretreatment, higher enzymatic hydrolysis yields could be

explained because cavitation effects caused by introduction of an ultrasound field into the enzyme processing solution greatly enhance the transport of enzyme macromolecules toward the substrate surface. Furthermore, mechanical impacts produced by the collapse of cavitation bubbles provide an important benefit of opening up the surface of solid substrates to the action of enzymes. The maximum effects of cavitation occur at 50^{O}C, which is the optimum temperature for many enzymes [13, 50-52].

Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) is a physicochemical pretreatment method for lignocellulosic biomass. The main aim of SPORL pretreatment method is to pretreat the wood chips in an aqueous sulfite or bisulfite solution followed by mechanical size reduction. The decrease of the strong recalcitrance of lignocellulosic biomass by SPORL is achieved by combined effects of dissolution of hemicelluloses, depolymerization of cellulose, partial delignification and partial solution of lignin. In SPORL pretreatment, degradation products such as furfural and HMF are produced lower than other pretreatment methods [53].

Liquid hot water (LHW) pretreatment is still being developed and indicates a great potential because of its simplicity, low generation of inhibiting byproducts and high yields. LHW is a hydrothermal pretreatment. In LHW, pressure is applied to maintain water in the liquid state at high temperature. Usually, temperature is between 170 - 230°C, pressure is higher than 5 MPa and retential time is between seconds and hours. In LHW pretreatment process, catalyst and chemicals are not required. The principle of this pretreatment method is treatment of lignocellulosic material by subcritical pressurized water, eventually assisted by CO_2 – enhanced hydrolysis. Geneally, LHW pretreatment method has cost saving potential due to low reactor cost, no catalyst cost. However, water demanding of process and energy requirement is higher. The industrial scale of LHW process has not been developed yet [6, 14].

Steam explosion (SE) is the most commonly used physicochemical pretreatment method for lignocellulosic materials. Steam explosion method is a hydrothermal pretreatment method in which biomass is treated with high pressure saturated steam then the pressure is decreased suddenly. This method makes biomass explosive decompression. As a physicochemical pretreatment, it combines mechanical forces

and chemical effects due to hydrolysis of acetyl groups which include in hemicellulose. Water acts as an acid at high temperatures in steam explosion method. Mechanical effects cause to separation of fibers due to the explosive decomposition because of suddenly pressure drop. In combination with the partial hemicellulose hydrolysis and solubilization, the lignin is redistribured and removed from the lignocellulosic material. When hemicellulose is removed, enzyme accessibility to the cellulose microfibrils is increased. Steam explosion includes two fractions. One of these fractions is liquid part which includes monomeric and oligomeric sugars mainly from hemicellulose solubilization. The other fraction is solid part which includes digestible cellulose and lignin. The initial temperature of steam explosion process is 160°C - 260°C and pressure is between 0.69 to 4.83 MPa. After a few seconds or few minutes pressure is decreased to atmospheric pressure. Optimal hemicellulose solubilization and hydrolysis can be achieved by both high temperature and short residence (270°C, 1 min) or lower temperature and longer residence time (190°C, 10 min). Recents studies show that lower temperature and longer residence time is more favorable. Due to high temperature, hemicellulose degradation and lignin transformation become, thus the potential of cellulose hydrolysis increases. The parameters that affect steam explosion process are residence time, temperature, biomass size and moisture content. On the other hand severity factor ($logR_0$) is important for steam explosion. Severity factor is shown in Equation 2.1.

$$logR_0 = \log\left(t \times e^{((T-100)/14.75)}\right) \quad \textbf{(2.1)}$$

Where t is time (minute) and T is temperature (°C). For maximum sugar yield, severity factor of steam explosion is should be between 3.0 and 4.5.

Higher severity factor causes an increase removal of hemicelluloses from the solid fraction and enhanced cellulose digestibility but also increases higher sugar degradation. Using moderate conditions should decreases sugar degradation and generation inhibitors. Furthermore, high severity factor causes decreasing the degree of polymerization and so lignin content in the solid fraction increases owing to cellulose solubilization.

Advantages of steam explosion pretreatment method are significantly lower environmental impact, lower capital investment, more potential for energy

efficiency, less hazardous process chemicals and conditions and complete sugar recovery. Moreover, using bigger chip size is available without acid catalyst, increasing hydrolysis yield in enzymatic hydrolysis and being feasible for larger scale production are the main advantages of steam explosion pretreatment. Although as it is stated that steam explosion without acid catalyst is an advantage, using acid catalyst has some advantages too. The addition of acid catalyst increase cellulose digestibility and improve hemicellulose hydrolysis so H_2SO_4 can be used as a catalyst for steam explosion pretreatment method. As a result of using acid as a catalyst is high equipment cost.

Steam explosion process requires less energy than mechanical comminution to achieve the same particle size reduction. The conventional mechanical methods require 70% more energy than steam explosion. Steam explosion is one of the most cost effective and successful pretreatment processes for wide range of raw materials such as poplar, eucalyptus, olive residues, corn stover, wheat straw, sugar bagasse, grasses and hemp [12].

It is suggested that two-step pretreatment is maximizing sugar recovery. In the first step, pretreatment is performed at a low temperature to solubilize the hemicellulose fraction and the cellulose fraction is subjected to a second pretreatment step at temperatures higher than 210°C. It causes higher ethanol yield and lower enzyme dosage during enzymatic hydrolysis. One other option is, combining steam explosion method with other pretreatment methods. Therefore, alkaline pretreatment, ILs pretreatment, organosolv pretreatment and milling pretreatment are an alternative to usage with steam explosion.

The main disadvantage of steam explosion is generating of some toxic compound derived from sugar degradation during pretreatment and enzymatic hydrolysis and fermentation step is affected for this disadvantage [9, 54-67]. Table 2.4 shows that summary of advantages and disadvantages of pretreatment methods [6].

Where H is high effect, M is moderate effect, L is low effect and 0 is no effect. As it is seen in Table 2.4, every pretreatment method has both advantages and disadvantages. All pretreatment methods should be comparing in Table 2.4.

Table 2.4 : Advantages and disadvantages of pretreatment methods [6].

Pretreatment Methods	Increase Accessible Surface Area	Cellulose Decrystallization	Hemicellulose Solubilization	Lignin Removal	Lignin Structure Alteration	Generation of Toxic Compound
Mechanical Comminution	H	H	0	0	0	0
Extrusion	H	H	0	-	-	-
Acid	H	0	H	M	H	H
Alkali	H	H	M/H	H	H	L
Organosolv	M	-	H	M/H	M	M/L
Ozonolysis	M	M	M/H	H	M	L
Ionic Liquids	M	H	H	M/H	M	M/L
Wet Oxidation	H	-	H	M	H	L
Microwave	H	H	L	H	H	L
LHW	H	-	H	L	M	L
AFEX	H	M	M	L	H	L
SPORL	H	M	H	M	M	L
CO_2 Explosion	M/H	-	M	H	M	M
Steam Explosion	H	-	H	M	H	H
Biological	M	0	0	H		0/L

2.8 Hydrolysis

Hydrolysis is converting cellulose molecules to six-carbon sugars (C6) and hydrolysis reaction is showed below.

$$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6 \quad \textbf{(2.2)}$$

As it is seen in Equation 2.2, cellulose is reacted with H_2O and C6 sugars are produced after hydrolysis step. After lignocellulosic materials hydrolysis, C6 sugars can result in yield of between 0.5 and 0.75 g/g celloluse. It is equal to 1000 g of cellulose converts to 500 – 750 g of C6 sugars [4]. Hydrolysis should be acidic hydrolysis and enzyme based hydrolysis.

2.8.1 Acid based hydrolysis

Dilute or concentrated sulfuric acid is used for acid based hydrolysis. The concentrated acid process for producing sugars from lignocellulosic biomass has a long history. The ability to dissolve and hydrolyze native cellulose in cotton using concentrated sulfuric acid followed by dilution with water was reported in the literature as early as 1883. The concentrated acid disrupts the hydrogen bonding between cellulose chains, converting it to a completely amorphous state. Once the cellulose has been decrystallized, it forms a homogeneous gelatin with the acid. The cellulose is extremely suitable to hydrolysis. Thus, dilution with water at modest temperatures provides complete and rapid hydrolysis to glucose, with small amount of degradation. Concentrated acid hydrolysis results in the release of fermentable sugars, however they are toxic, corrosive, and hazardous and require reactors that are resistant to corrosion. Because of these conditions process is become very expensive. More environment friendly and economically feasible techniques for deriving sugars from lignocellulosic biomass are prefered so dilute acid hydrolysis followed by enzymatic hydrolysis is one of them. Dilute acid hydrolysis has also been successfully developed for pretreatment, and it highly increases the efficiency of the enzymatic hydrolysis step. Sulfuric acid concentration below 4% is generally used as it is comparatively inexpensive and helps in achieving high reaction rates. Since sugar decomposition takes place at moderate temperature, this process requires a high temperature and neutralization of pH is also necessary for the downstream enzymatic hydrolysis or fermentation process. Apart from this, to make the process economically feasible, these acids must be recovered from the reaction mixture after hydrolysis [6, 68].

2.8.2 Enzyme based hydrolysis

Enzymatic hydrolysis is carried out by cellulase enzymes which are highly specific, and the products of the hydrolysis are usually reducing sugars including glucose.

Unlike chemical hydrolysis, enzymatic hydrolysis is conducted at moderate conditions at a pH of 4.8 and temperature of 45-50°C, these conditions are ideal for the cellulase enzyme. The main advantage of enzymatic hydrolysis over chemical hydrolysis is that it does not generate a corrosion problem But the process takes several days whereas it is only a few minutes in the case of chemical hydrolysis. Moreover, the final product of enzymatic hydrolysis inhibits the enzyme and ultimately affects the process unless they are removed immediately after they are formed. A main drawback of lignocellulosic ethanol production is the cost of the enzymes [6,69]. For enzymatic hydrolysis of lignocellulosic materials uses cellulose most commonly produced by soft-rot fungi such as *Trichoderma sp*, *Penicillum sp* and *Aspergillus sp* [4].

Enzymatic hydrolysis of cellulose includes three steps. These are adsorption of cellulases to the surfaces of cellulose, hydrolysis of cellulose to glucose and desorption of cellulases. Presence of non-cellulose component such as lignin and hemicellulose and high crystallinity of cellulose make the adsorption of cellulase a rate-limiting step. One of the main factors that affect the yield and initial rate of enzymatic hydrolysis of cellulose is substrate concentration. An increase of substrate concentration causes an increase of yield and reaction rate of hydrolysis at low substrate levels. For reducing the inhibition of hydrolysis, several methods have been developed. These methods are using high concentration of enzymes, the supplementation of β-glucosidases during hydrolysis and removal of sugar during hydrolysis [6].

2.9 Fermentation of Sugar

The general requirements of an organism for ethanol production from pentose sugar hydrolysate should be high ethanol yield, high productivity, good tolerance against inhibitors as well as high ethanol concentrations and ability to ferment at relatively low pH. The potential yield of ethanol from lignocellulosic materials varies significantly between feedstocks, because of varieties of their component content [4].

The conversion of fermentable sugars (C5 sugars and C6 sugars) into bioethanol by the metabolism of microorganisms, as represented by the Equation 2.3 and Equation 2.4 [6].

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2 \quad \textbf{(2.3)}$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad \textbf{(2.4)}$$

S. cerevisiae is one of the most commonly used yeasts for ethanol fermentation using glucose. However, it does not have the ability of fermenting pentose sugars. The most promising yeast species identified so far for the pentose fermentation are, *C. shehatae, P. stipitis* and *P. Tannophilus*. Fermentative yeasts generally possess both aerobic and anaerobic pathways along with adaptive regulatory mechanisms.

Theoretically, ethanol yield is maximum 95% after fermentation of C5 and C6 sugars [4]. Although the theoretical yield is 0.511 g of ethanol/g hexose, maximum real yield is around 0.485 g ethanol/ g hexose and 0.488 g CO_2 [6, 70].

The calculation of ethanol yield is is given in Equaiton 2.5;

$$\text{Bioethanol yield} = M \times TY \times GR \times FE \quad \textbf{(2.5)}$$

Where M is mass of biomass, TY is theoretical yield, GR is Glucose Recovery and FE is fermentation efficiency so [71];

Bioethanol from cellulose = cellulose mass × 0.5111 × 0.76 × 0.75

Bioethanol from hemicellulose = hemicellulose mass × 0.5175 × 0.90 × 0.50

For applying these formulas, cellulose mass and hemicellulose mass should be determined.

2.10 Distillation of Bioethanol

For increasing ethanol concentration some procedures are needed as distillation. The distillation unit is composed of distillation columns, reboilers which are located in the bottom of the columns and condensers in the top of the columns. Before distillation, fermented blend usually contains 7 - 7.5 % of bioethanol (w/w). This blend enters first column for a primary seperation. This column contains 35-45% (w/w) ethanol vapor and these are directed to send second column for a primary distillation. Then, mixture is sent to rectification column. The residues of distillation are defined as vinasse and represent an environmental problem because for producing 1 L of bioethanol 15 L of vinasse is generated. [6].

2.11 Bioethanol in the World

As a leader engine biofuels, bioethanol has wide application in the world. The history of bioethanol as an engine fuel has come from internal combustion engines. In 1897, N.A. Otto had used ethanol in his engine study. In 1900's, Henry Ford had defined that ethanol – gasoline blend will have been fuel for future. Since 1970's, the attention to bioethanol has increased depends on increasing to price of fossil fuels. Figure 2.9 shows that bioethanol production in the world [1,72].

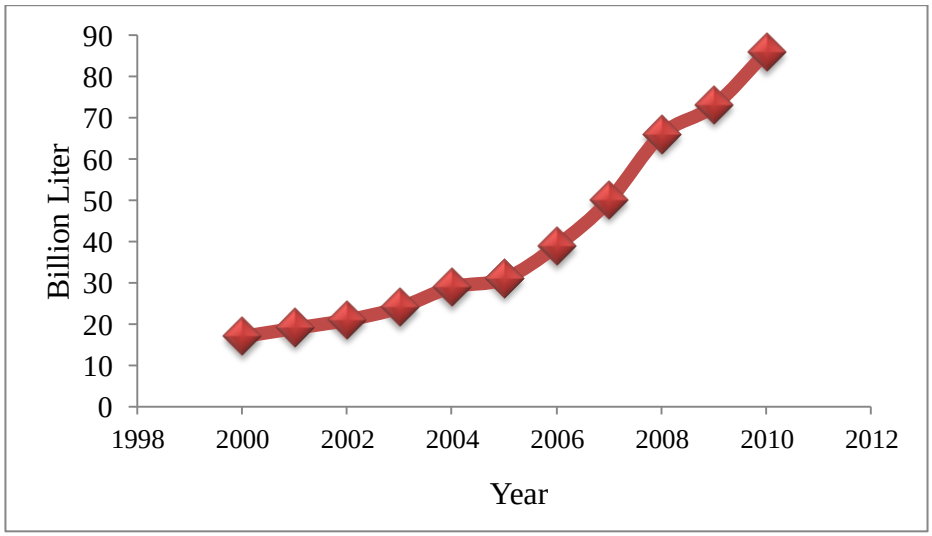

Figure 2.9 : Bioethanol production in the world [73].

As it is seen in Figure 2.9, bioethanol production is high effective increasing between 2000 and 2010. While bioethanol production was nearly 20 billion liters in 2000, in 2010 bioethanol production is increased to 86 million liters. In Table 2.5, amount of bioethanol productions by countries are given.

In Table 2.5, countries are sorted by amount of bioethanol production in 2010. As it is seen in Table 2.5, USA is the first country for bioethanol production and bioethanol production is 49,440 million liters in 2010. Then Brazil, China, France, India, Canada and Germany are following the USA. These countries have more than 1000 million liters bioethanol production. In Thailand, UK, Spain, Russia, Ukraine, Argentina, Colombia, Poland, Indonesia, South Korea and Italy, bioethanol is produced between 1000 to 100 million liters. World total bioethanol production is 101,370 million liters in 2010, 87,703 million liters in 2009 and 78,885 million liters

in 2008. It is equal to bioethanol production was increased by 10.05% in 2009 and 13.48% in 2010.

Table 2.5 : Amount of ethanol production in the world (million liters) [8,74].

Country	**2008**	**2009**	**2010**
USA	35.315	41.072	49.440
Brazil	22.551	27.165	28.680
China	4.050	4.450	7.000
France	1.545	1.850	1.938
India	2.075	1.725	-
Canada	900	1200	1.500
Germany	689	1.040	1.120
Thailand	396	700	795
UK	350	580	650
Spain	438	570	620
Russia	559	529	544
Ukraine	370	400	370
Argentina	212	250	345
Colombia	283	325	342
Poland	198	400	270
Indonesia	190	250	250
South Korea	175	175	172
Italy	118	115	110
Other Countries	9.516	4.907	5.374
Total	**78.885**	**87.703**	**101.370**

In United States, biofuels production and consumption are supported by government. In 2006, August 3, 250 Million $ budget is provided for Research and Development (R&D) projects. According to “New Energy Law”, 2006, target is defined as 7.5 million gallons of biofuels consumption in 2012 and USA hit their target in 2008. New target is 130 billion liter/year of biofuels consumption in 2017 [8].

In many states in the USA, bioethanol is currently used a gasoline additive. In the last 6 years, bioethanol has taken place of methyl tertiary butyl ether as a gasoline additive and it causes reduce air pollution. In US cars, mostly used blend in E10 and 95% of bioethanol is manufactured from corn starch. After 1988, all car engines are designed to run on with E10 and in most cases it is up to E20. Currently, at least 7 million cars have engines which can use an 85% ethanol blend in the USA [6].

According to “Development of Biofuel Marketing Draft Law”, 2011 January, target is 50 % of car which will be produced in 2015 and 90 % of car which will be produced in 2016 should run with E85. Due to only bioethanol sector, 53.6 billion dollars value-added and 36 billion dollars revenue growth are provided in 2010. Also employment is generated for 400,677 people. On the other hand, decreasing 445 million barrels of oil for oil import is generated and it equals to 34 billion dollars saving [8]. Table 2.6 shows that, bioethanol production capacity and number of biorefinery in the USA.

Table 2.6 : Bioethanol production and biorefinery number in the USA [8].

	Biorefinery	Capacity
2000	54	6746
2001	56	7264
2002	61	8872
2003	68	10231
2004	72	11721
2005	81	13773
2006	95	16931
2007	110	20765
2008	139	29818
2009	170	39952
2010	189	44897

As it is seen in Table 2.6, according to 2000, number of biorefinery in 2010 is increased 3 times. However, bioethanol capacity is increased 7 times in the USA.

Brazil is the one of the most ethanol producer in the world. First of all, bioethanol had used as a gasoline additive (5%) in 1931. Legal Regulation had done about bioethanol in 1938. Blend ratio of bioethanol – gasoline mixture had increased to 22% in 1993. At present blend ratio is between 20 – 26% for depends on bioethanol prices. In Brazil, the feedstock of bioethanol is mostly sugarcane and more than 80% of vehicles are running with bioethanol. Even though, small airplane engines are being developed. The reason of sugarcane was the chosen substrate, is its great adaptation to Brazilian soil and weather conditions. 1 million people are working in bioethanol sector in Brazil. 340 bioethanol plants are being and it causes 186 billion dollars saving. According to Brazilian Sugarcane Industry Association (UNICA), it is expected that bioethanol plants will increase to 409 and bioethanol production will reach to 35.7 billion liters [6,8].

Because of security of energy supply, agricultural development and challenging with global warming, biofuels are significant for EU. It is intended that according to "White Paper" in 1997, 5 million tones liquid biofuels (biodiesel and bioethanol) will be used in 2020. Also, according to "Green Paper" (2000), "Biofuel Encouragement Directive" (2003), "Kyoto Protocol" (2005), "Biofuel Strategy Paper" (2006), some policies have developed about biofuel production and consumption. For this reason, biofuel consumption is legal obligation for most European countries. According to "Vision 2030 Paper" which has prepared by Comission of EU, it is aimed that 25% biofuels will be consumed in 2030. On the other hand, since 2010, second generation of biofuels production will be commercialized and biorefining will be applied in 2020. In 2020 it is aimed that, 20% of energy consumption will be produced by renewable energy and 10% of biofuel is consumed [8].

Firstly, bioethanol took place in 5015 – Petroleum Marketing Law as "Blending production with Gasoline" in 2003 and uses as gasoline additive according to TSE EN 228 which is standard of automotive gasoline. Bioethanol which is producing from domestic resources and blended up to 2 percent with gasoline has zero Special Consumption Tax. According to "Technical Regulation Paper" which is reported by Energy Marketing Regulatory Authority (EPDK) in September 2011, in 2013, it is obligated that blend ratio of bioethanol/gasoline will be minimum 2% and in 2014, this ratio will increase to 3% [75].

Production, usage and selling of alcohol in Turkey are regulated and controlled by Republic of Turkey, Tobacco and Alcohol Market Regulatory Authority (TAPDK). In Turkey, there are 4 plants for bioethanol production. Total bioethanol production capacity in Turkey is 184 million liters [74]. Konya Sugar Incorporated Company produce 84 million liters per year and feedstocks are sugar beet and molasses [8]. Tarkim (Agricultural Chemical Technologies Incorporated Company) is placed in Bursa and feedstocks are wheat and corn [76]. The production capacity of TARKIM is 40 million liter/year. Tezkim (Tezkim Incorporated Company) is placed in Adana, feedstock is corn and production capacity is 40 million liters [77]. In Eskisehir Sugar Factory, bioethanol production capacity is 20 million liters. Bioethanol should be produced according to ASTM D 4806 and Turkish Standards Institution (TSE) [1,3,75].

2.12 Literature Review

In this part other studies about pretreatment technologies on different woody biomass, different pretreatment technologies of bioethanol production are given.

Zhu et al (2008) investigate SPORL for robust enzymatic saccharification of pine. The results indicated that after the SPORL pretreatment of pine chips with 8–10% bisulfite and 1.8–3.7% sulfuric acid on oven dry wood at 180°C for 30 min, more than 90% cellulose conversion of substrate was achieved with enzyme loading of about 14.6 FPU cellulase after 48 h hydrolysis. Glucose yield from enzymatic hydrolysis of the substrate per 100 g of untreated pine wood (glucan content 43%) was about 37 g (excluding the dissolved glucose during pretreatment) [53].

Hideno et all (2009) make a research about wet disk milling (WDM), a continuous pretreatment to enhance the enzymatic digestibility of rice straw. Glucose and xylose yields by wet disk milling, ball milling, and hot-compressed water treatment were 78.5% and 41.5%, 89.4% and 54.3%, and 70.3% and 88.6%, respectively. Wet disk milling and hot-compressed water treatment increased sugar yields without decreasing their crystallinity was compared with conventional ball milling and hot-compressed water treatment [78].

Silva et al (2010) investigate the effectiveness of ball milling and wet disk milling on treating sugarcane bagasse and straw were compared. Glucose and xylose hydrolysis yields at optimum conditions for BM-treated bagasse and straw were 78.7% and 72.1% and 77.6% and 56.8%, respectively. Maximum glucose and xylose yields for bagasse and straw using WDM were 49.3% and 36.7% and 68.0% and 44.9%, respectively. BM improved the enzymatic hydrolysis by decreasing the crystallinity, while the defibrillation effect observed for WDM samples seems to have favored enzymatic conversion. Bagasse and straw BM hydrolysates were fermented by Saccharomyces cerevisiae strains. Ethanol yields from total fermentable sugars using a C6-fermenting strain reached 89.8% and 91.8% for bagasse and straw hydrolysates, respectively, and 82% and 78% when using a C6/C5 fermenting strain [79].

Chen and Xu (2010) make a research about a pretreatment method coupling steam explosion with alkaline peroxide for wheat straw. After the complex pretreatment, the cellulose content in wheat straw increased from 31.5% to 67.2%. In the hydrolysate of wheat straw pretreated with the complex method and steam explosion,

the glucose concentration was 110.9 g/L and 67.8 g/L, respectively. The optimal conditions for SSF were 40 8C, 120 h, cellulase loading 40 FPU/(g wheat straw), yeast inoculum10% (v/v) and substrate concentration 16.7% (w/v). Under the optimal conditions, the total ethanol concentration in SSF of wheat straw pretreated with steam explosion and alkaline peroxide reached 51.5 g/L, and an overall yield of 81.1% was obtained [80].

Öhgren et all (2006) investigate that steam-pretreated corn stover at 5, 7.5 and 10% water-insoluble solids (WIS) with 2 g/L hexosefermenting Saccharomyces cerevisiae (ordinary compressed baker's yeast). SSF at 10% WIS resulted in an ethanol yield of 74% based onthe glucose content in the raw material and an ethanol concentration of 25 g/L. Neither higher yeast concentration (5 g/L) nor yeast cultivated on the liquid after the pretreatment resulted, under these conditions, in a higher overall ethanol yield [81].

Chen et al (2011) investigate The combined pretreatment of rice straw using dilute-acid and steam explosion followed by enzymatic hydrolysis and compared with acid-catalyzed steam explosion pretreatment. In addition to measuring the chemical composition, including glucan, xylan and lignin content, changes in rice straw features after pretreatment were investigated in terms of the straw's physical properties. The effect of acid concentration on the acid-catalyzed steam explosion was studied in a range between 1% and 15% acid at 180°C for 2 min and also investigated the influence of the residence time of the steam explosion in the combined pretreatment and the optimum conditions for the dilute-acid hydrolysis step in order to develop an integrated process for the dilute-acid and steam explosion. The optimum operational conditions for the first dilute-acid hydrolysis step were determined to be 165°C for 2 min with 2% H2SO4 and for the second steam explosion step was to be carried out at 180°C for 20 min; this gave the most favorable combination in terms of an integrated process. It is found that rice straw pretreated by the dilute-acid/steam explosions had a higher xylose yield, a lower level of inhibitor in the hydrolysate and a greater degree of enzymatic hydrolysis; this resulted in an increase in the overall sugar yield when compared to the acid-catalyzed steam explosion [82].

Cara et al (2007) assest the production of fuel ethanol from olive tree pruning. Olive tree pruning was submitted to steam explosion pre-treatment in the temperature range

190–240°C, with or without previous impregnation by water or sulphuric acid solutions. The influence of both pretreatment temperature and impregnation conditions on sugar and ethanol yields was investigated by enzymatic hydrolysis and simultaneous saccharification and fermentation on the pretreated solids. Results show that the maximum ethanol yield (7.2 g ethanol/100 g raw material) is obtained from water impregnated, steam pretreated residue at 240°C. Nevertheless if all sugars solubilized during pre-treatment are taken into account, up to 15.9 g ethanol/100 g raw material may be obtained (pre-treatment conditions: 230°C and impregnation with 1% w/w sulphuric acid concentration), assuming theoretical conversion of these sugars to ethanol [83].

EXPERIMENTAL STUDY

The aim of experimental study is measuring how pretreatment methods affect on sugar conversion from lignocellulosic materials such as pine wood. All experiments were done in University of Idaho (UI), Biological and Agricultural Department, James Martin Laboratory (JML) in USA.

3.1 Materials

In experimental study, Northern US Pine Wood is used as feedstock. This wood is unshelled and being as a timber. It is harvested in 2010. All experiments are done with this pine wood. In Figure 3.1, the Northern US Pine Wood is shown.

Figure 3.1 : A photograph of northern US pine wood.

First of all, pine wood is prepared for experiments. Then, the wood is shaved with chopper two times in JML. These shaved particles of pine wood are called sawdust (SD) in experimental study and the sizes of these particles are smaller than 10 mesh which is equal to 2 mm. As it is seen in Figure 3.2 that, after first shaving pine woods are converted pine shavings and after second shaving pine woods are converted to pine sawdust.

Figure 3.2 : Pine wood, wood shavings and sawdust.

All chemicals which were used in experiment are analytical purity. Deionized water (DI) is produced from laboratory condition in JML. Sulfuric acid (H_2SO_4), Sodium metabisulfite, D-Mannose and Glucose which was bought as Dextrose, Anhydrous are provided from EMD Chemical Company and Cellulase Enzyme (*Trichoderma Reesei*) and D(+)galactose are provided from Sigma. 3,5 Dinitrosalicylic acid, sodium hydroxide (NaOH) and hydrochloric acid (HCl) are provided from Sigma-Aldrich. Phenolphthalein indicator and citric acid monohydrate are provided from Fisher Scientific. Myo-inositol, lead (II) carbonate ($PbCO_3$), IR 120 H^+ resin, IR 402 OH^- resin which are seen in Figure 3.12 are provided from Alfa Aesar Chemical Company. Rochelle salts (Sodium potassium tartrate) is provided from VWR, solid Phenol is provided from ACROS and L-arabinose is provided from BD Bioscience.

Figure 3.3 : IR 120 H^+ resin and IR 402 OH^- resin.

3.2 Methods

Experimental part is divided by 3 main groups as defining physical properties, pretreatments and sugar determination. Experiments were done as shown in Figure 3.4.

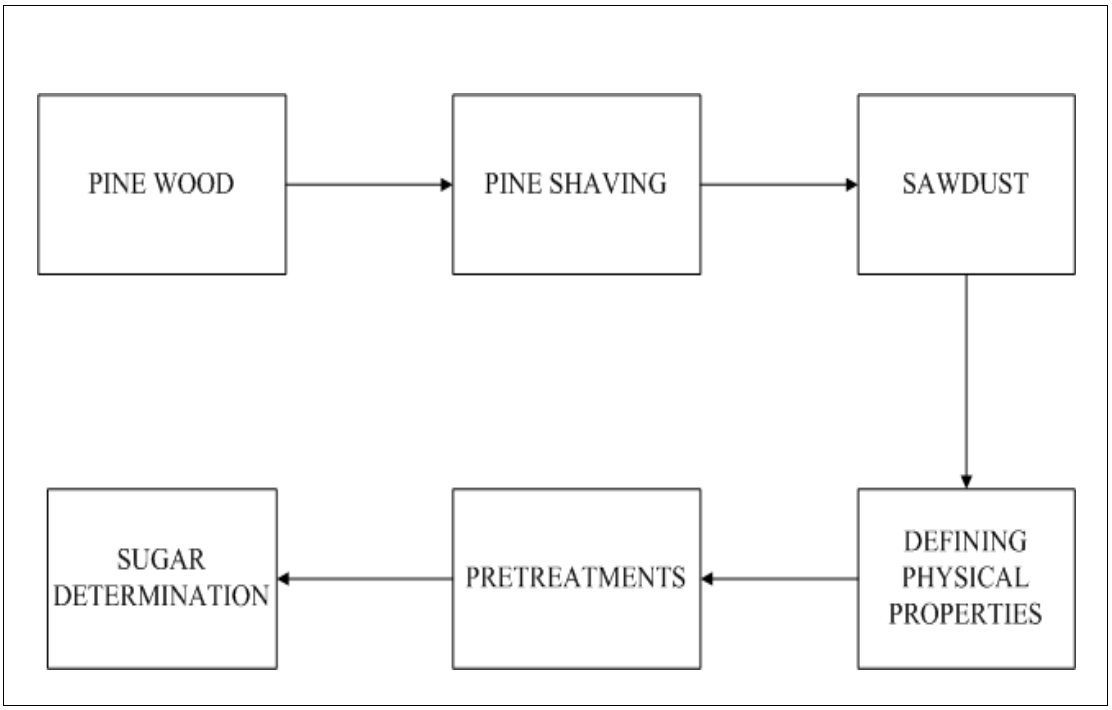

Figure 3.4 : General concept of experiment.

As it is seen in Figure 3.4, pine wood is used as feedstock than it is shaved and it is becomes pine shavings. Because of producing SD, pine shavings are shaved again. SD is analyzed for defining physical properties then pretreatments methods are applied on SD. At last, sugar determination is detected for all samples to measure effects pretreatment methods on glucose conversion.

3.2.1 Defining physical properties of SD

Moisture content, ash content, lignin content and sugar content of sawdust is defined as physical properties. In Figure 3.5, the flow diagram of all physical analysis is shown. The moisture content of SD is measured then left for air drying. After air drying, moisture content is measured again. Also lignin analysis and ash analysis is done after air drying. Lignin analysis is done after ball milling and steam explosion pretreatments.

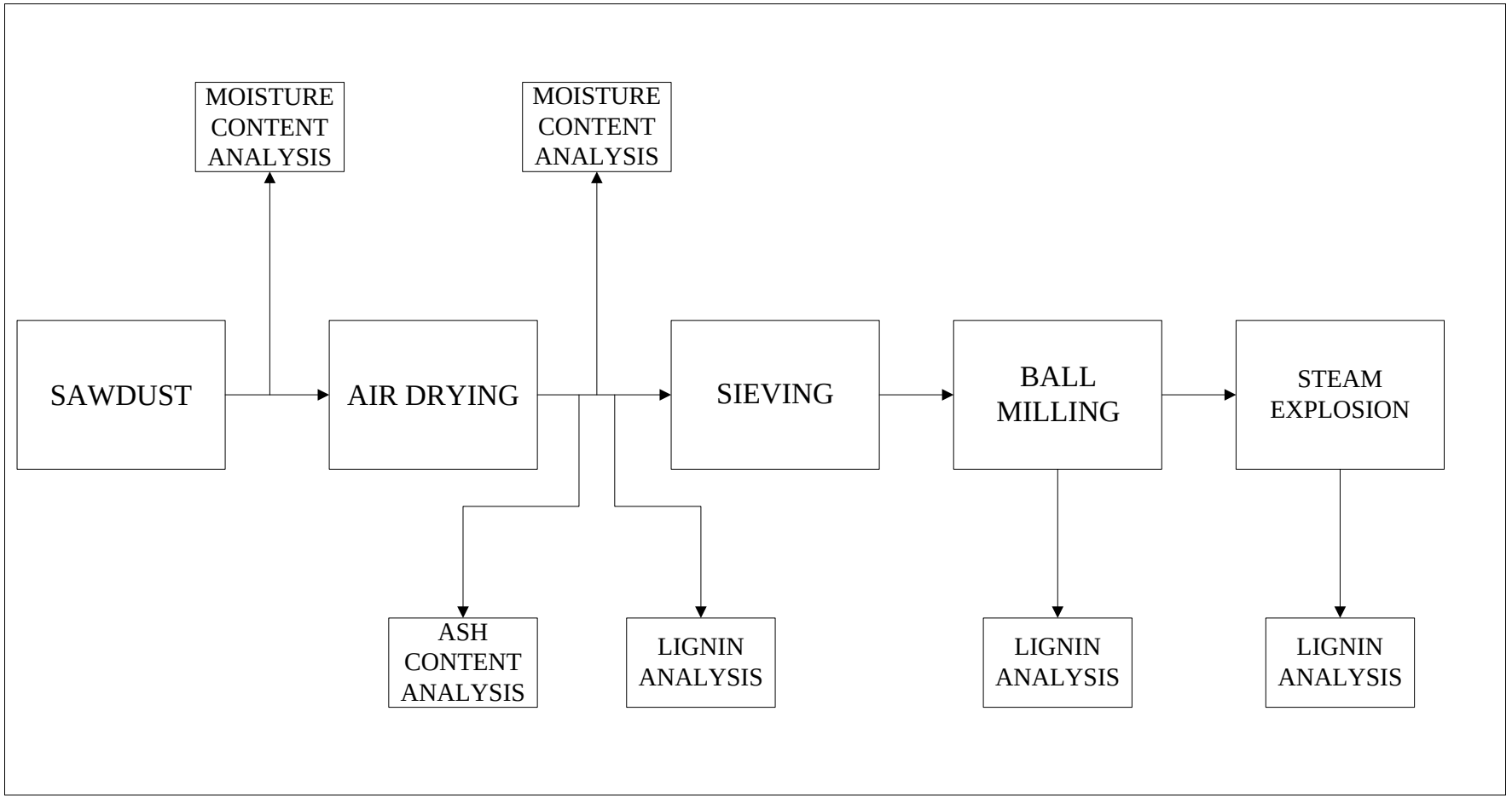

Figure 3.5 : Defining physical properties of SD.

Portions of moisture content procedure are similar to ASTM E1756-01 and T412 om-02. For moisture content, sawdust is measured two times. First of all, sawdust sample is taken and measured moisture content. Then, it is left 5 days for air drying and measured again. For measuring moisture content, dry glass dishes is weighted and placed in oven at 105°C for drying for four hours. The dishes are taken from oven and placed in dessicator after 4 hours. The dishes are weighted again after cooling and placed back in oven. Weights are recorded. Then exact 2 grams of sawdust is weighted and placed into the weighing dish. The weight of the sample plus weighing dish is recorded. The sawdust is placed in an oven at 105°C for four hours. The sawdust is removed from the oven and allowed it to cool to room temperature in a desiccator. The dish containing the oven-dried sample is reweighted. The sawdust is placed back into a convection oven at 105°C to reach constant weight. Constant weight is defined as ± 0.1% change in the weight percent solids upon one hour of re-heating the sample [84].

Ash content procedure is substantially similar to ASTM Standard Method Number E1755-01 "Standard Method for the Determination of Ash in Biomass". First of all, crucibles are numbered with a porcelain marker and placed in the muffle furnace at 600°C for four hours. Then the crucibles are removed from the furnace and put directly in dessicator. Cooling time is recorded and the crucibles are reweighted. Then the sawdust is placed in crucibles and put in muffle furnace at 600°C. First aim is measure constant weight which is defined as less than ± 0.3 mg change in the weight upon one hour of re-heating the crucible. Sawdust is measured 0.5 to 2 mg and placed in the tarred crucible. The weight is recorded. Crucibles are put in muffle furnace and the temperature is set 600°C. The crucible is placed over flame until smoke appears using an ashing burner and clay triangle with stand. The smoke is ignited and waited the sample to burn until no more smoke or flame appears. Crucibles are placed in the muffle furnace at 600°C for 24 hours. After waiting time, the crucibles are removed from the furnace and placed directly in dessicator and left to cool for a specific amount of time which is equal to the initial cool time of the crucibles. The crucibles with ash are measured again and weights are recorded. Then, sawdust are placed back to muffle furnace at 600°C for one hour. Then cooled them again and reweighed. If weight difference between 2 results are smaller than 0.3 mg, the ash content is measured exactly [85].

Portions of lignin content procedure are substantially similar to ASTM E1758-01 "Standard method for the Determination of Carbohydrates by HPLC For lignin content, the sample is weighted 200 mg and set in a small test tube. Then 2 mL, 72% sulfuric acid is added and incubated for 60 mins at 30^{o}C with regular stirring using a glass rod. This part is primary hydrolysis step. Hydrolysis step is sown in Figure 3.6.

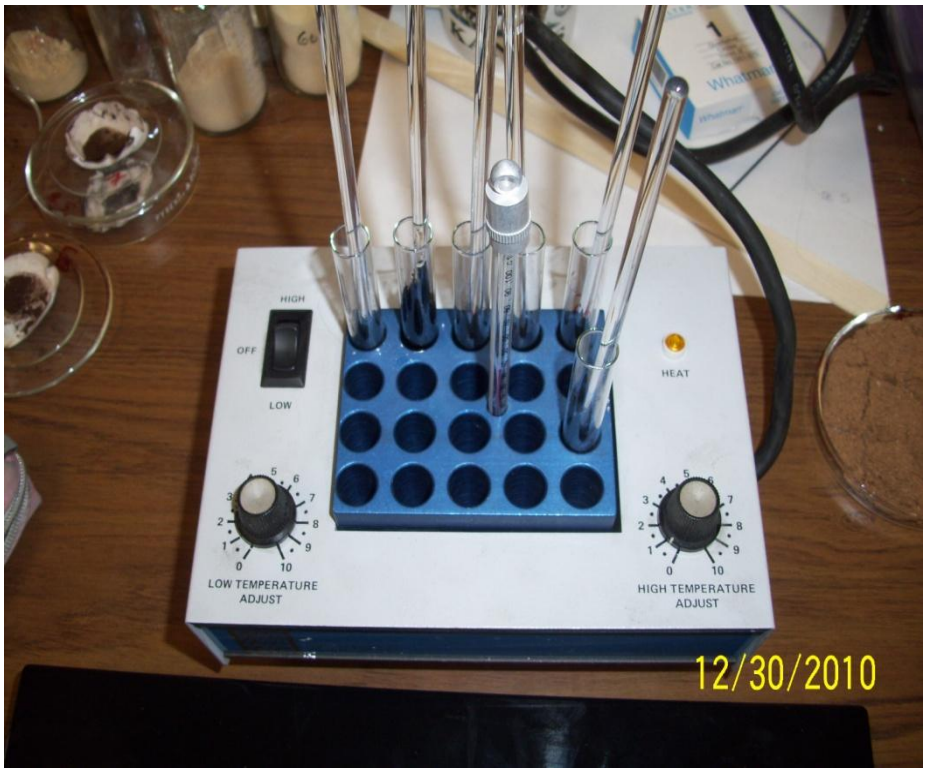

Figure 3.6 : Hydrolysis step at lignin analysis of SD.

Then primary hydrolyzate is transferred into a 200 mL Erlenmeyer flask with the aid of distilled water (56 mL, final sulfuric acid concentration will be v/v 4%) and cover the flask with a small beaker. Flask is placed in a pressure cooker and heat until starts to hiss and from then time for 30 mins. This part is secondary hydrolysis step. Then the cooker is let cool down slowly. On the other hand, glass crucibles with a glass fiber mat are preweighed. The secondary hydrolyzate is filtered through the crucibles and washes with 40 mL of distilled water. The filtrate is transferred to a 200 or 250 mL volumetric flask and rinse flask with distilled water (80 mL) and the flask is let to flask reach room temperature and make up to the line. Then store in fridge for one week. For collecting lignin, set the crucible in an oven over night at 104^{o}C. The crucible is cooled in a desiccator for 10 mins and weighed again. Collected lignin is shown in Figure 3.7 [86].

Figure 3.7 : Lignin of SD.

3.2.2 Pretreatments

In this section, ball milling and steam explosion pretreatments are detailed. First of all sawdust is taken for pretreatment experiment then it is sieved with different size of sieves. Working time of sieve is 10 minutes with 180 rpm. The sizes of sieves are 35, 42, 65, 75, 100, 115 and 125 mesh size. Millimeter conversion of mesh sizes are given in Table 3.1. After sieving, sawdust is collected 7 different size groups however only 35 mesh sized sawdust is selected because the amount of 35 mesh sized sawdust (SD35) are more than others.

Table 3.1 : Conversion of mesh sizes to millimeter.

Mesh Size	mm
35	0.425
42	0.325
65	0.212
75	0.200
100	0.150
115	0.125
125	0.120

3.2.2.1 Ball mill pretreatment

In the ball mill pretreatment, Rancimat Retsch PM100 is used for mechanical communition. As it is shown in Figure 3.8, in ball mill pretreatment, SD35 is used. 10 grams of SD35 is taken and set in ball mill machine. The speed of ball mill machine is 200 rpm. There are 25 metal balls are in machine and the diameters of balls are 12 mm. This experiment time periods are 2, 4, 8, 12, 16 and 24 hours. After ball mill pretreatment, samples are put in sieve again. Working time of sieve is 10 minutes with 180 rpm. SD35 is divided 7 size groups after sieving. The sizes of treated particles are between 35 and 125 meshes. These are called ball milled samples and they are showed as BMx. X is shown that the size of ball milled sample which shown as Table 3.2.

Figure 3.8 : Ball mill machine.

Table 3.2 : Abbreviations of BM samples.

Sizes	**Ball Milled**	**Ball Milled with Enzymatic Hydrolysis**
35 mesh	SD	SDH
42 mesh	BM42	BM42-H
65 mesh	BM65	BM65-H
75 mesh	BM75	BM75-H
100 mesh	BM100	BM100-H
115 mesh	BM115	BM115-H
125 mesh	BM125	BM125-H

3.2.2.2 Steam explosion pretreatment

After ball mill pretreatment, steam explosion is done. BM100, BM115 and BM125 have high sugar conversion however BM75 is lower than others. Both increasing to sugar conversion of BM75 and obtaining BM75 is not difficult as BM100, BM115 and BM125, BM75 is used for steam explosion experiment. For doing steam explosion, exact measure of sawdust is taken. BM75 is put in 200 mL DI water and heated at 230°C and waited 15 minutes. Then, solid – liquid mixture is put in a reactor which is resisted high pressure and temperature. Reactor is heated up 190°C and mixer speed is 1000 rpm and waited 10 minutes. Pressure is given as psig which is pound per square inch (gage pressure - psig). The pressure of reactor is between 230 to 260 psig. The valve is open suddenly and pressure is decreased to atmospheric pressure. Liquid part of mixture is collected in a vessel and includes some solid particles. This liquid part is filtered and collected solid particles are washed with DI water and filtered with vacuum, dried in an oven for a night 104°C. The solid particles which stay in reactor are collected, washed with DI water and filtered with vacuum, dried in an oven for a night at 104°C. In this pretreatment, variable parameters are temperature, quantity of sawdust, nitrogen pressure, releasing time and valve condition. Parameters which used in steam explosion are given in Table 3.3.

Table 3.3 : Parameters in steam explosion.

No	Temperature (°C)	Pressure (psig)	Quantity of Sawdust (g)	Nitrogen Pressure (psig)	Releasing Time (sec)	Valve Condition
1	190	242	4	0	27	Fully open
2	190	238	4	64	23	Fully open
3	190	235	4	64	22	Fully open
4	250	611	4	64	26	Fully open
5	190	230	4	64	47	360° open
6	190	244	8	64	20	Fully open
7	190	235	4	64	21	Fully open
8	190	261	8	64	52	360° open
9	190	231	4	0	22	Fully open
10	190	236	8	0	25	Fully open

As it is seen in Table 3.3, all experiments except number 4 run at 190 °C. The reason of this is sample which was heated at 250 °C was burnt. Then, it is decided that experiments were continue with 190 °C. The severity factor of steam explosion in this study is 3.65.

In Figure 3.9, filtering with vacuum is shown at left side of the figure and reactor of steam explosion is shown at the right side of the figure. After vacuum filtering liquid part which is seen in Figure 3.9 is called liquid part of SE and shown as SxL where x is number of experiment. Wood particles which filtered with vacuum is steam exploded samples and shown as SxV and SxR where x is number of steam explosion experiment, V is samples which was collected from vessel after steam explosion and R is samples which was collected from reactor after steam explosion. Table 3.4 is shown that name of samples.

Figure 3.9 : Experimental set-up for SE.

Table 3.4 : Abbreviation of samples after SE.

Experiment Number	**Collected from Vessel**	**Collected from Reactor**	**SxV + enzymatic hydrolysis**	**SxR + enzymatic hydrolysis**	**Collected as Liquid**
1	S1V		S1V-H		S1L
2			S2V-H		S2L
3	S3V		S3V-H	S3R-H	S3L
4	Sample was burnt because of high temperature				
5	S5V	S5R	S5V-H	S5R-H	S5L
6	S6V	S6R	S6V-H	S6R-H	S6L
7	S7V	S7R	S7V-H	S7R-H	
8	S8V	S8R			S8L
9	S9V	S9R			S9L
10	S10V	S10R			S10L

3.2.3 Sugar determination

After pretreatment methods, the sugar conversion of samples should measure. For determining sugar, 2 procedures are applied. One of is cellulose enzyme activity, other is HPLC. The difference between cellulose enzyme activity and HPLC is cellulose enzyme activity measures total amount of sugar, HPLC determines individual sugars. In Figure 3.10, experiment diagram at sugar determination is showed.

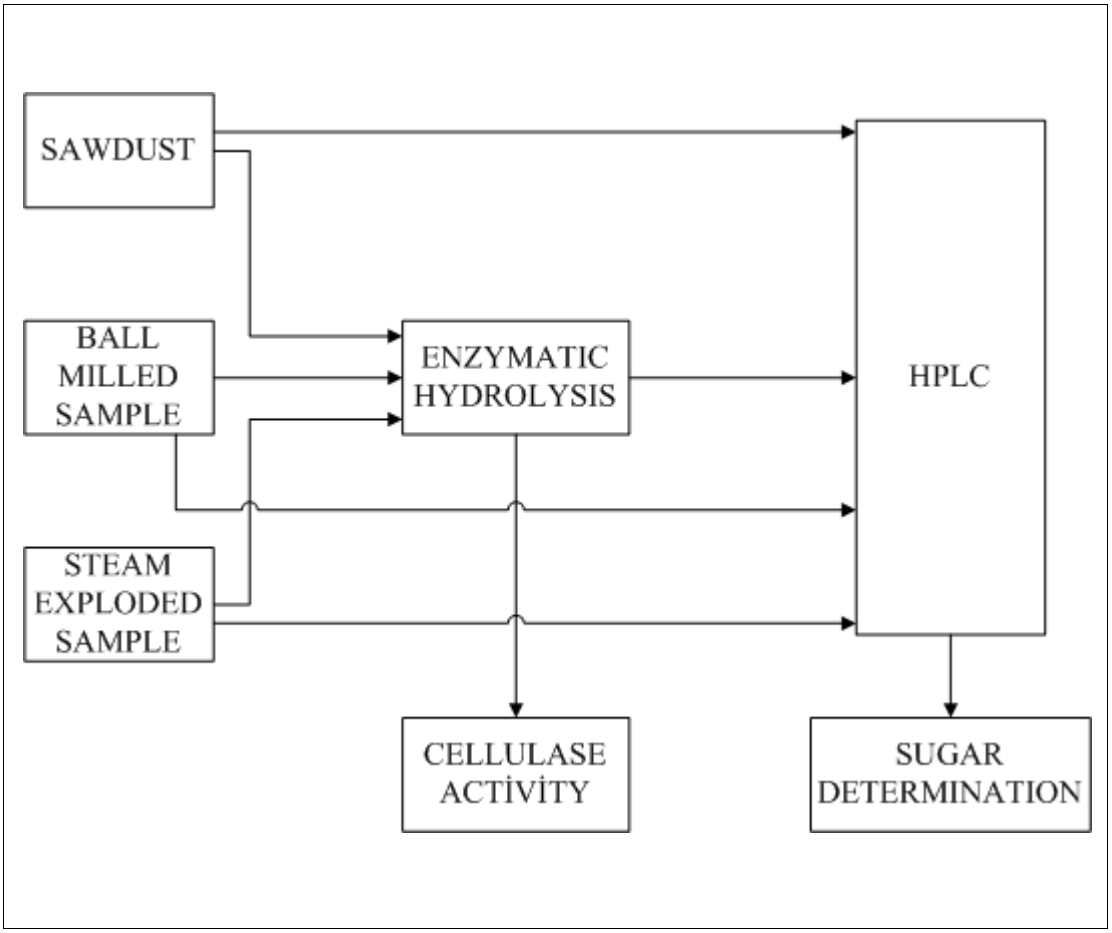

Figure 3.10 : Experiment diagram at sugar determination.

In this section, saeudst, ball milled and steam exploded samples divided to two groups. First group of samples are directly prepared for HPLC and sugar determination analysis is done. It is for how much sugar includes at these samples before enzymatic reaction. Then, second group of samples are hydrolyzed with enzyme. After enzymatic hydrolysis, cellulase activity of these samples is measured. At last, all hydrolyzed samples are prepared for HPLC and sugar determination analysis is done.

In enzymatic reaction, Cellulase (Trichoderma Reesei) is used as an enzyme. Enzymatic hydolysis method describes a procedure for measurement of cellulase activity using International Union of Pure and Applied Chemistry (IUPAC) guidelines. The procedure has been designed to measure cellulase activity in terms of "Filter Paper Unit" (FPU) per milliliter of undiluted enzyme solution [12].

In this procedure, first of all DNS Reagent is prepared. For preparing DNS reagent, 1416 mL distilled water, 10.6 grams of 3,5 Dinitrosalicylic acid and 19.8 grams of Sodiumhydroxide are dissolved then 306 g of Rochelle salts (sodium potassium tartrate), 7.6 mL of Phenol (melt at 50° C) and 8.3 g of Sodium metabisulfite are added. 3 mL sample is titrated with 0.1 N HCl to the phenolphthalein endpoint. It should take 5-6 mL of HCl.

After DNS reagent, citrate buffer is prepared. For Trichoderma reesei which is the enzyme used in hydrolysis step, cellulase assays are carried out in 0.05 M citrate buffer pH 4.8. For other cellulase enzymes, the pH and the assay temperature may be different. 210 g citric acid monohydrate, 750 mL DI water and 50 – 60 g NaOH are mixed in a baker. NaOH is added until pH equals exact 4.3. Then the solution is diluted 1 L and pH is checked again. If necessary, NaOH can be added until the pH is 4.5. When the 1 M stock citrate buffer stock is diluted with water to 50 mM the pH should be 4.8.

In enzymatic hydrolysis, enzyme concentration is prepared to be 4 unit/mL. Enzyme is diluted with 50 mM citrate buffer solution. 400 mg of sample are taken from all samples and put in plastic tubes. Then 40 mL enzyme solution is added on it. Plastic tubes which include sample and enzyme solution are put in orbital shaker bath which is shown in Figure 3.11. Bath temperature is 50°C and speed is 300 rpm. Hydrolysis experiment takes 72 hours. In every 24 hour, 4 mL sample is taken from tubes and sugar quantity is measured with micro-plate filter paper method.

For preparing glucose standards, a working stock solution of anhydrous glucose (10 mg/mL) should be made up. Aliquots of this working stock should be tightly sealed and stored frozen. The release of glucose equivalents is determined using a Standard curve produced using glucose samples having concentrations of 0, 2, 4, 6, 8, 19 mg/mL. 50 mM of citrate buffer is used to make glucose standards.

Figure 3.11 : Enzymatic hydrolysis tubes in orbital shaker bath.

The detection of glycosidic bond cleavage by this method involves the parallel and identical treatment of three categories of experimental tubes (assay mixtures, blanks and controls, and glucose standards), prepared as detailed below. The substrate is a 50 mg Whatman No. 1 filter paper strip. The filter paper is cut into 7 mm circles using a Standard hole punch. In this procedure, only 10 samples can be analyzed at a time because incubation occurs in a Standard thermocycler which can hold up twelve Polymerase Chain Reaction (PCR) tube strips. For each sample to be analyzed, a 7 mm filter paper circle is added to the first four PCR tubes in a strip. These four will be used to measure cellulose degradation. The second set of four PCR tubes is served as a sample blank. The significant point is all paper circles must be at the bottom of the tubes.For glucose standards, 6 PCR tubes are set in strip without filter paper. 4 PCR tubes with filter paper circles are set in a strip for negative control. 40 µl of citrate buffer is added all samples including those used to standards and controls. 20 µl citrate buffer is added to four negative control reaction tubes. 20 µl of glucose standard is added to other tubes. 20 µl of sample is added to all 8 PCR tubes. Figure 3.12 is shown that the strip photograph which is ready for analysis. This strip includes samples, glucose standards and negative controls.

Figure 3.12 : PCR tubes on a strip.

In Figure 3.12, numbered PCR tubes between 1 and 6 are samples which will be analyzed. The tubes which are numbered to 0 to 10 are glucose standards. N labeled tubes are defined as negative control tubes. Figure 3.13 is shown that the application of samples in test tubes.

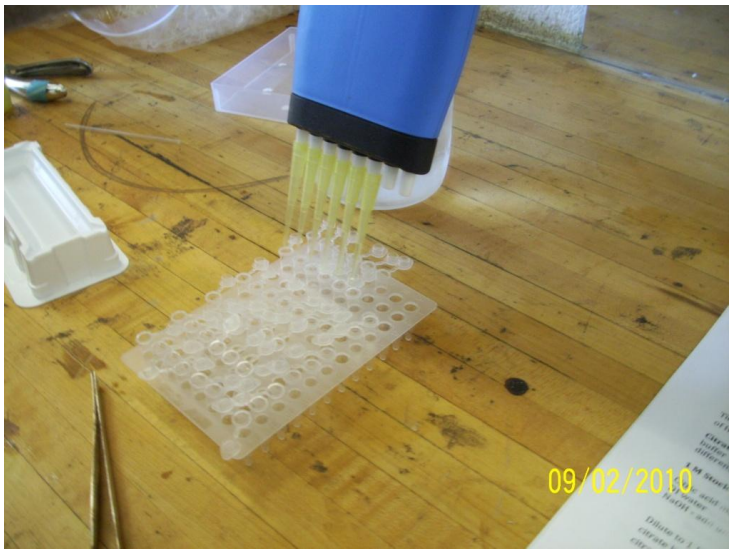

Figure 3.13 : The application of samples.

After preparing PCR test tubes, samples are incubating at 50 °C for 1 hour in the thermocycler. Then it is cooled 4 °C and stopped reaction by adding 120 µl of DNS reagent to each tube including standards and negative controls. The strip is put in thermocycler again and heated at 95 °C for 5 minutes. Tubes are cooled to 4 °C again and waited 5 minutes. Figure 3.14 is shown above that a photograph of thermocycler.

Figure 3.14 : Photograph of thermocycler.

The color of samples after thermocycler changes from yellow to red. It is depend on glucose concentration. High concentration of glucose samples turn red. In Figure 3.15, color changes are seen.

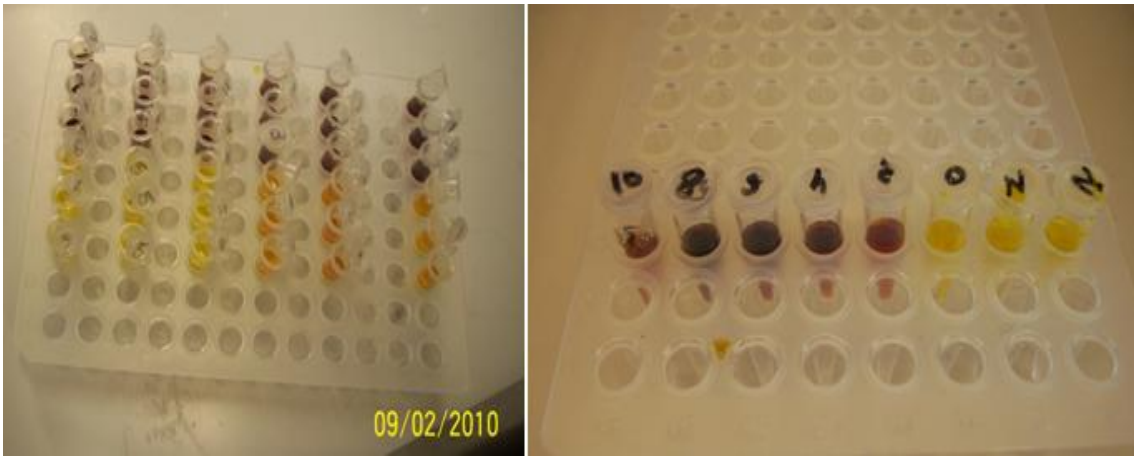

Figure 3.15 : Color changes after thermocycler.

After taking samples from thermocycler, 36 µl of each samples are taken and put in 96 well microplate. This microplate is containing 160 µl of DI water. Pipette is up and down for mixing. In this step, the bubbles should not be in plate. Then the plate is put in photospectrometer and results are taken [87].

For measuring sugar content, HPLC is used. For HPLC analysis, Phenomenex columns are used. All samples are prepared for HPLC as detailed below.

Glucose, galactose, arabinose, xylose and mannose are determined in this analysis. Before doing HPLC analysis, lignin analysis should be done because in HPLC analysis, secondary filtrate is used which hydrolyzed in lignin analysis. First of all, volumetric flask containing secondary filtrate hydrolysis is left to reach room temperature. Then 5 mL of filtrate is taken using a pipette and placed in a small test tube and added inositol (1 mL, at 0.5 mg/mL concentration) as an internal standard and mix well. After this process, 0.161 g of lead carbonate ($PbCO_3$) is added and mixed. The solution should give a neutral pH. If it is too acidic, some more lead carbonate is added. All samples are centrifuged 5 minutes and transferred to a plastic syringe pass through a small (pre-rinsed with water) ion exchange cartridge (2 mL plastic syringe containing 0.5 mL of IR120 H+ resin and 0.5 mL IR402 OH- resin). The supernatant is filtered through a 0.45mm syringe filter and the filtrate is collected into a HPLC vial. The vial is labeled. All samples are prepared for HPLC. Preparing standard solution, 10.0 mg glucose, 5.0 mg mannose, 5.0 mg galactose, 5.0 mg xylose, 5.0 mg arabinose and 5.0 mg inositol are mixed and diluted 100 mL with DI water. The solution is filtered as samples via a syringe into a HPLC vial. In Figure 3.16, HPLC sample and machine are showed. For preventing errors, 2 standards are used in analysis. In experimental study xylose analysis wasn't done [12].

Figure 3.16 : HPLC sample and HPLC.

HPLC is the Waters Breeze system. Samples and standards are placed into the HPLC auto sampler and the analyzer is set up. The columns are two Phenomenex columns for carbohydrates operating at 90°C with a flow of 0.5 mL/min. Injection volume is 30 mL. A Refractive index detector is used for the non-chromophoric sugars. Data analysis is done through the Breeze software package.

RESULTS AND DISCUSSIONS

Results are given as physical properties of sawdust, pretreatments and sugar determination.

4.1 Physical Properties of Sawdust

Physical properties are moisture, ash and lignin content. Moisture and ash content of sawdust are given in Table 4.1 and lignin content of sawdust is given in Table 4.2. Moisture content of sawdust was measured before and after air drying. According to Table 4.1**Table**, moisture content was decreased from 4.38 % to 2.40 % after air drying and it is equal to 1.98 %. Ash content of sawdust is measured after air drying and it is equal to 0.18 %. As it is seen in Table 4.2 that lignin analysis is done for all samples which include sawdust, ball milled samples and steam exploded samples. According to Table 4.2, lignin content of sawdust is 32.25 %. Lignin content of ball milled samples is between 24% and 29% and lignin content of steam exploded samples is between 27% and 35%. Lignin content of steam exploded samples which collected from vessels are higher than samples which collected from reactors. It is seen that lignin ratio of steam exploded samples are higher than other samples because after steam explosion some sugars are collected from liquid part so lignin ratio of solid part is increased.

4.2 Ball Mill Pretreatments

Although pretreatment methods include BM and SE as defined on method section, SE results are given after enzymatic hydrolysis and HPLC analysis. In Table 4.3 shows ball mill results.

Table 4.1 : Moisture and ash content of SD (w/w, %).

Sample	Moisture Content	Ash Content
Before air drying	4.38	
After air drying	2.40	0.18

Table 4.2 : Lignin content of all sawdust samples (w/w, %).

Sample	**Lignin**
SD	32.25
BM35	28.85
BM42	27.4
BM65	26.85
BM75	26.20
BM100	25.55
BM115	24.15
BM125	29.7
S1V	27.7
S3V	33.85
S5V	34.45
S5R	30
S6V	33.6
S6R	28.55
S7V	33.15
S7R	27.15
S8V	36.2
S8R	34
S9V	33.2
S9R	32.3
S10V	35.65
S10R	34

Table 4.3 : Ball mill results after 2, 4, 8, 12, 16 and 24 hours (w/w, %).

	Time (h)						
Sample	**0**	**2**	**4**	**8**	**12**	**16**	**24**
SD	100	17.85	7.32	1.37	0.31	0.21	0
BM42	0	3.79	3.56	0.63	0.31	0.53	0
BM65	0	27.38	18.82	2.74	1.44	2.74	1.61
BM75	0	42.97	45.37	48.10	36.05	24.16	0.90
BM100	0	2.67	0.20	0.74	0.82	1.16	0.20
BM115	0	1.74	5.70	10.76	3.71	3.80	9.54
BM125	0	3.59	19.02	35.65	57.36	67.41	87.75
Total	100	100	100	100	100	100	100

It is seen in Table 4.3 that, when time is zero, the ratio of SD is 100%. After 2 hours, 82.15 % of SD was converted to BM42, BM65, BM75, BM100, BM115 and BM125. After 4 hours, SD ratio was only 7.32 %. BM65, BM75 and BM125 are more than 15 % in 4 hours. In 8 hours, SD ratio was decreased to 1.37 %. Ratios of BM75 and BM125 were increased. In 12 hours, BM75 was decreased to 36.05 % and

BM125 were increased to 57.36 %. In 16 hours, was decreased to 24.16 % and BM125 were increased to 67.41 %. At the last of ball mill experiment which was hour 24, 87.75 % of SD was converted to BM125.

4.3 Sugar Determination

In this section, cellulase activity results are given first, and then HPLC results are given. Figure 4.1 is shown that cellulase activity for all types of sawdust. The samples are taken at 1, 2, 4, 6, 7.5, 12, 22, 24, 48 and 72 h.

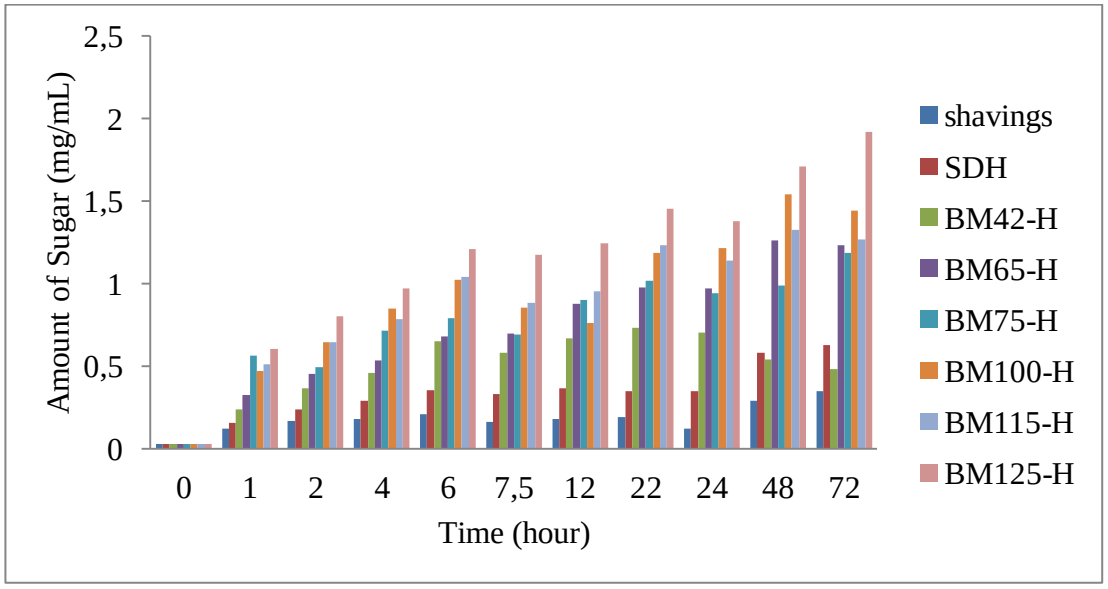

Figure 4.1 : Total sugar determination (1st run).

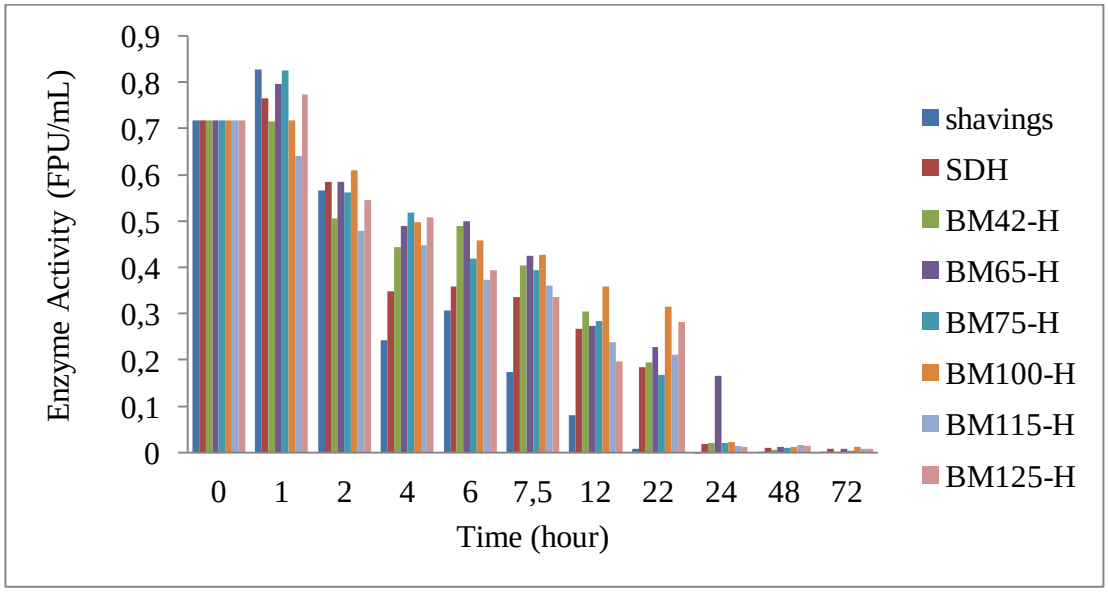

Figure 4.2 : Enzyme activity after 72 hours (1st run).

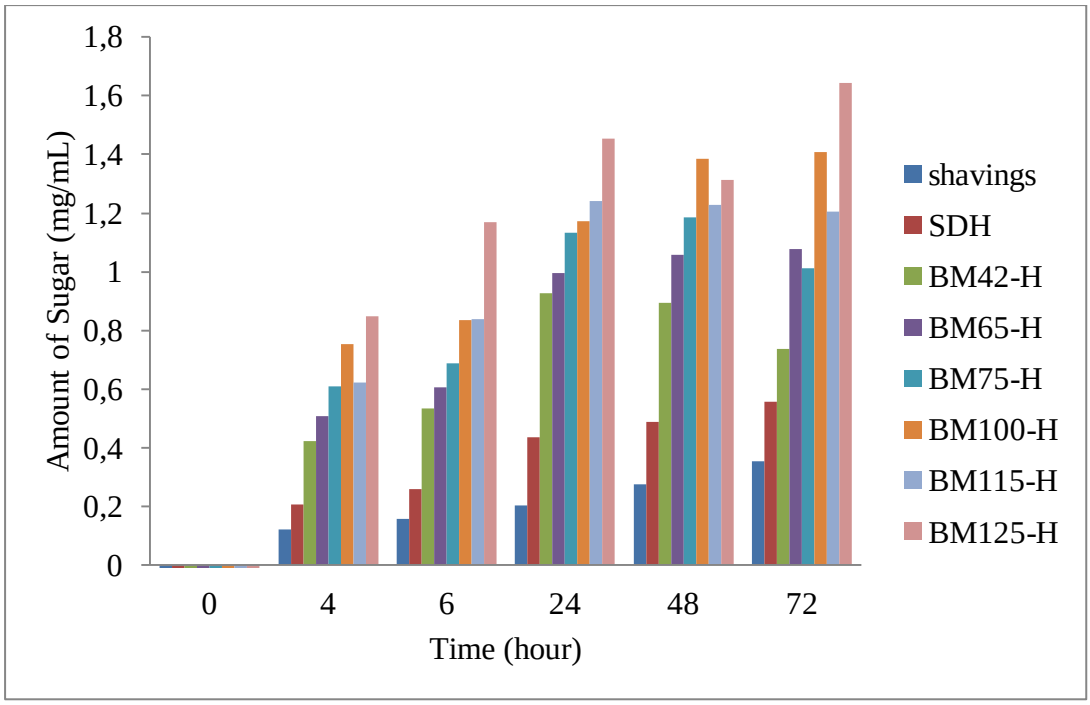

Figure 4.3 : Total sugar determination for BM samples (2nd run).

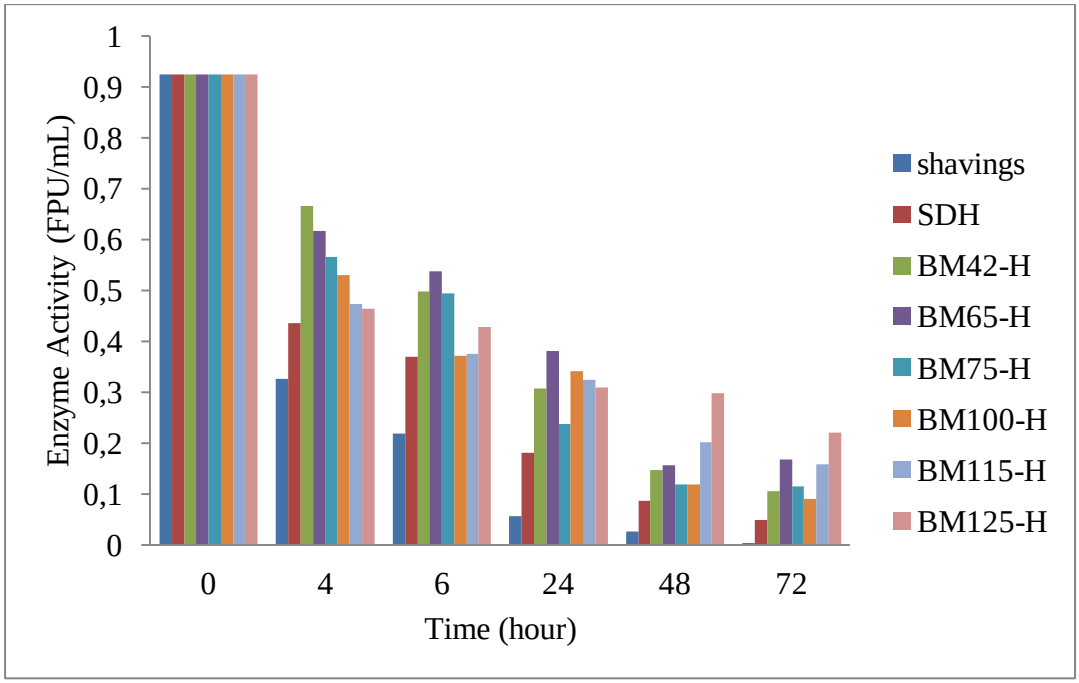

Figure 4.4 : Enzyme activity after 72 hours (2nd run).

Figure 4.1 and Figure 4.3 show that the amount of sugar at the end of enzymatic hydrolysis. Figure 4.2 and Figure 4.4 show that the enzyme activity at the end of enzymatic hydrolysis. It is clearly seen in Figure 4.1 and Figrue 4.3 that, amount of sugar increases with smaller wood particles. In two figures, the amounts of sugar in BM125 are 1.65 mg/mL and 1.92 mg/mL at the end of 72 hours. In BM115, they are

1.20 mg/mL and 1.26 mg/mL. In BM100, they are 1.40 mg/mL and 1.44 mg/mL and in BM75, they are 1.01 mg/mL and 1.18 mg/mL. Table 4.4 shows that, sugar conversion of sawdust.

Table 4.4 : sugar conversion of samples.

	Run 1 (mg/mL)	Run 2 (mg/mL)	Average (mg/mL)	Solution (mL)	Sample (mg)	Total Sugar (mg)	Conversion (w/w, %)
Shaving	0.346	0.355	0.350	40	400	14.007	3.50
SD	0.625	0.556	0.591	40	400	23.628	5.91
BM42	0.480	0.737	0.609	40	400	24.346	6.09
BM65	1.229	1.076	1.153	40	400	46.110	11.53
BM75	1.185	1.011	1.098	40	400	43.910	10.98
BM100	1.439	1.407	1.423	40	400	56.923	14.23
BM115	1.264	1.204	1.234	40	400	49.376	12.34
BM125	1.920	1.642	1.781	40	400	71.234	17.81

As it clarified in Table 4.4, total sugar conversion of BM125 is 17.81 %, BM115 is 12.34 %, BM100 is 14.23 %, BM75 is 10.98 %, BM65 is 11.53%, BM42 is 6.09 %, SD is 5.9 % and shaving is 3.5%. According to Table 4.4, cellulose enzyme activity depends on sample size. BM125, BM115 and BM100 are high conversion; however conversion of BM75 had decreased. Both BM75 have lower conversion than BM100, BM115 and BM125; and according to Table 4.3, BM75 are produced more than other samples with ball mill, BM75 was chosen as a feedstock of steam explosion pretreatment. Figure 4.5 and Figure 4.6 show that amount of sugar and enzyme activity for steam exploded samples after enzymatic hydrolysis.

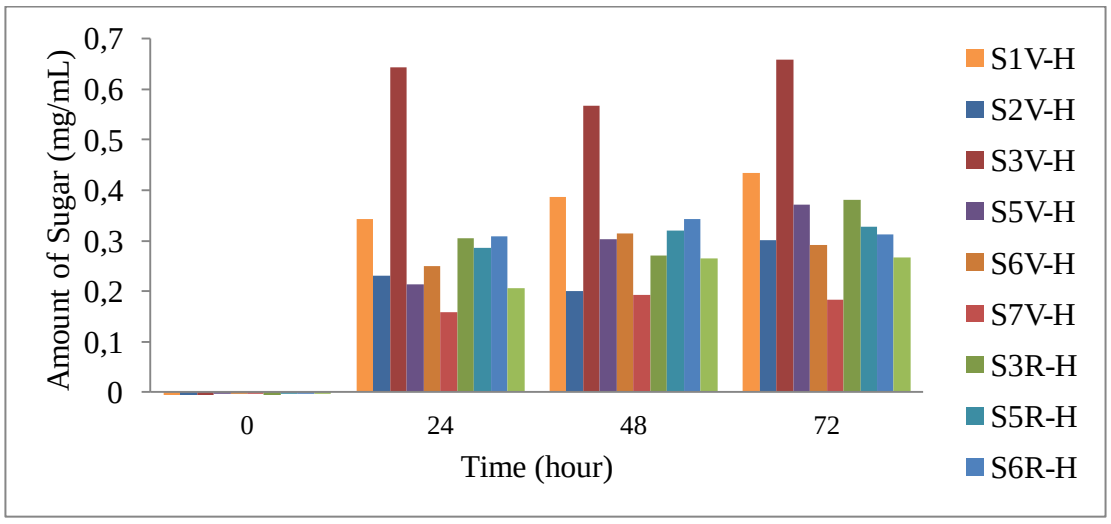

Figure 4.5 : Total sugar determination for SE samples.

According to Figure 4.5, most conversion was become at experiment 3 (S3V). Other results are not regularly distribution. HPLC results are shown more specific.

After this point, HPLC results are given. All samples are prepared for HPLC and sugar determination is measured. Table 4.5 is given below and detailed the sugar obtaining after 72 hour enzymatic hydrolysis.

Figure 4.7 is shown that sugar obtaining after enzymatic hydrolysis. First column is untreated sawdust which includes all size of sawdust. Second column in sawdust is hydrolyzed with enzyme in 72 hours.

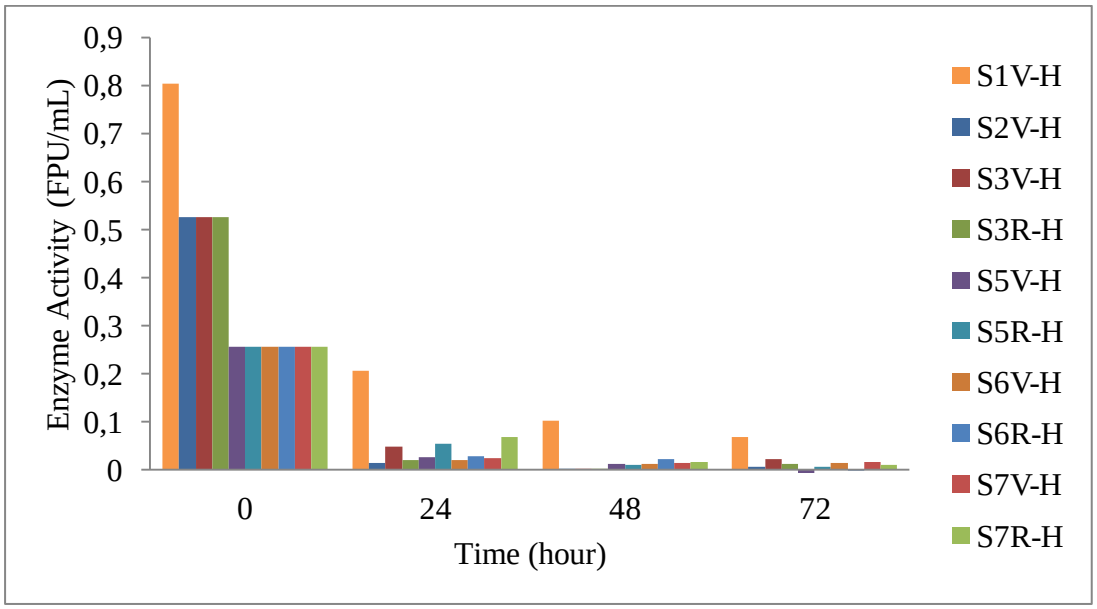

Figure 4.6 : Enzyme activity after 72 hours (Steam Exploded Samples).

Table 4.5 : Before and after enzymatic hydrolysis % conversion of sawdust sample.

Sugars	**Amount of Sugar (mg/mL)**		**Conversion (%) (w/w)**		**Obtaining(%)**
	SD	**SDH**	**SD**	**SDH**	
Glucose	0.2035	0.0150	25.44	0.15	0.59
Mannose	0.0516	0.0000	6.45	0.00	0.00
Galactose	0.0313	0.0080	3.91	0.08	2.05
Arabinose	0.0000	0.0000	0.00	0.00	0.00

Sawdust samples includes only 35 mesh sized samples without ball mill pretreatment. H means after enzymatic hydrolysis. For enzymatic hydrolysis, 400 mg of sawdust is blended with 40 mL solution (w/w, 1%) and for HPLC, 200 mg of sawdust is blended with 250 mL solution (w/w, 80%). Calculations are doing as considering these ratios. It is shown in Table 4.5 that, amount of glucose is 25.44 %

mannose is 6.45 % and galactose is 3.91 % in SD. After enzymatic hydrolysis only 0.15 % of glucose and 0.08% of galactose was obtained. Without pretreatments, only 0.59 % of glucose and 2.05 % of galactose were obtained. Cellulose activity results and high performance liquid chramotgraphy results should not compare because xylose sugar did not determined.

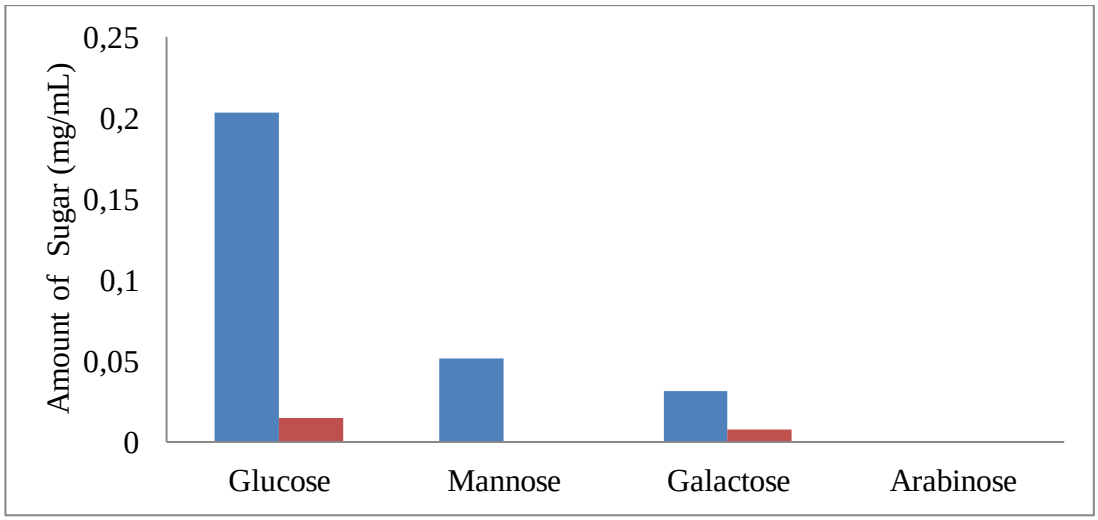

Figure 4.7 : Sugar determination of sawdust.

In Figure 4.7, sugar determination of sawdust is given. Blue bar shows sawdust after hydrolysis and red bar shows before hydrolysis. It is clearly seen in Figure 4.7 that, amount of glucose is higher than other sugars. It is desirable for lignocellulosic bioethanol production because glucose is converted bioethanol more than mannose, galactose and arabinose.

Other ball milled sample results are given in Figure 4.8. As seen in Figure 4.8 that, glucose is obtained more than mannose, arabinose and galactose. According to Figure 4.8, 100,115 and 125 mesh sized particles include more glucose than other size particles. It is depend on small sized particles' surface area is greater than bigger sized particles and enzyme could be more effective in small sized particles. This result is proved that ball mill pretreatment is effective for bioethanol production because of getting lignocellulosic biomass particles smaller.

Table 4.6 and Table 4.7 show HPLC results for ball milled samples. In Table 4.6, amount of glucose and mannose are given and in Table 4.7, amount of galactose and mannose are given. First column for each sugar is shown as mg/mL, second column is shown conversion ratio.

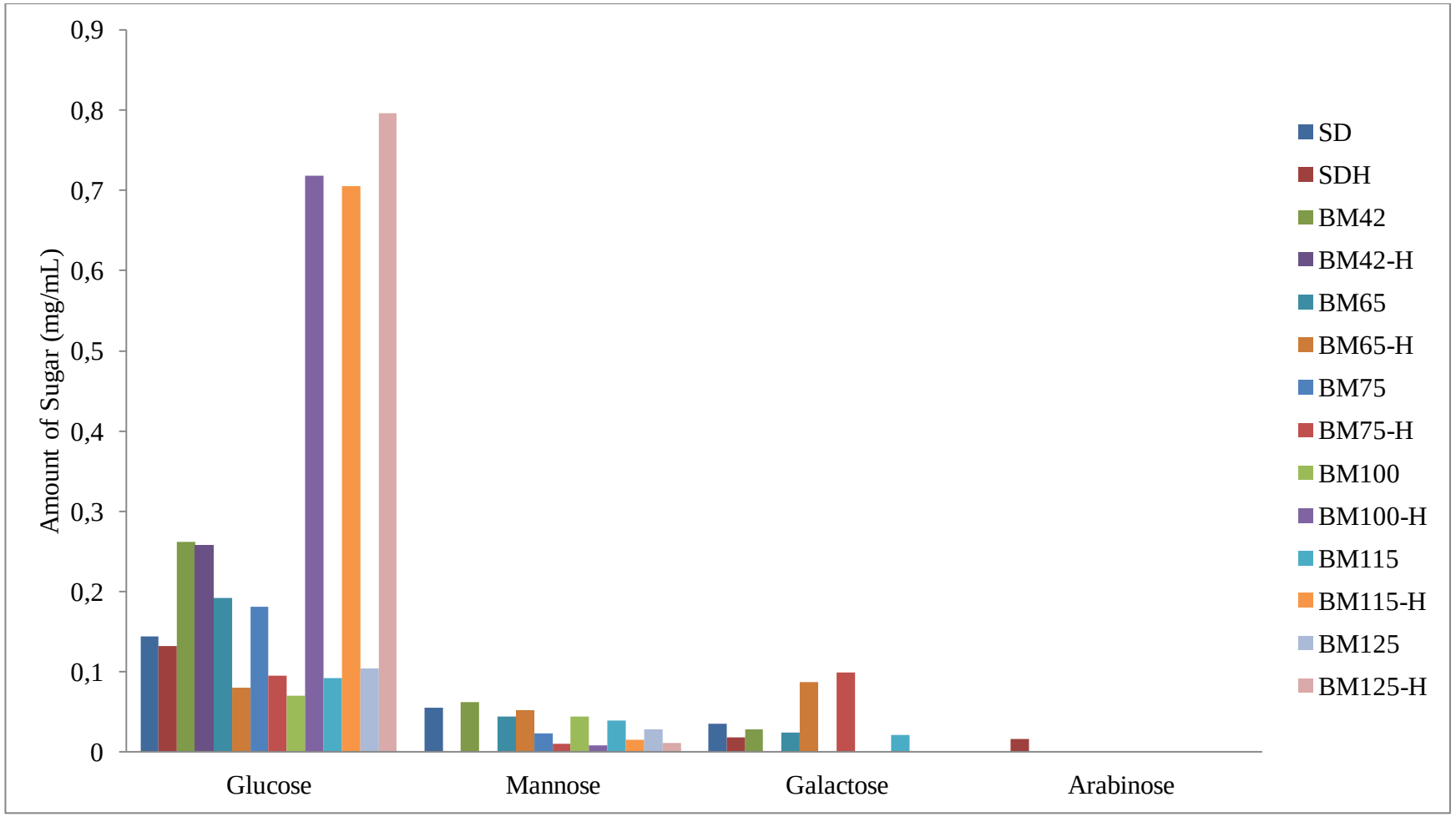

Figure 4.8 : Sugar determination for 35, 42, 65, 75, 100, 115, 125 mesh size with ball mill and hydrolysis pretreatment.

Table 4.6 : Amount of glucose and mannose.

Samples	Glucose		Mannose	
	mg/mL	(w/w),%	mg/mL	(w/w),%
SD	0.145	18.06	0.055	6.90
SDH	0.132	1.31	0	0
BM42	0.262	32.74	0.063	7.83
BM42-H	0.258	2.58	0	0
BM65	0.192	24.03	0.045	5.59
BM65-H	0.081	0.8051	0.052	0.52
BM75	0.181	22.62	0.024	2.96
BM75-H	0.095	0.95	0.01	0.10
BM100	0.07	8.76	0.044	5.50
BM100-H	0.719	7.19	0.009	0.09
BM115	0.092	11.50	0.039	4.92
BM115-H	0.705	7.05	0.016	0.16
BM125	0.104	13.01	0.028	3.48
BM125-H	0.796	7.96	0.011	0.11

Table 4.7 : Amount of galactose and arabinose.

Samples	Galactose		Arabinose	
	mg/mL	(w/w),%	mg/mL	(w/w),%
SD	0.036	4.44	0	0
SDH	0.018	0.18	0.017	0.17
BM42	0.028	3.50	0	0
BM42-H	0	0	0	0
BM65	0.024	3.05	0	0
BM65-H	0.087	0.87	0	0
BM75	0	0	0	0
BM75-H	0.099	0.99	0	0
BM100	0	0	0	0
BM100-H	0	0	0	0
BM115	0.021	2.67	0	0
BM115-H	0	0	0	0
BM125	0	0	0	0
BM125-H	0	0	0	0

In Figure 4.9, ratios of sugar components before and after enzymatic hydrolysis are given.

According to Figure 4.9, glucose conversion is higher than other sugars. For BM42, only 7.88% of glucose was obtaining after enzymatic hydrolysis. BM65 ratio is 3.35 %, BM75 ratio is 4.21 %. However; BM100 ratio is 82.07 %, BM115 ratio is 61.31

% and BM125 ratio is 61.20 %. Conversion of BM100, BM115 and BM125 are good enough for bioethanol production and it is explained that ball mill pretreatment is adequate for 100, 115 and 125 mesh sized particles.

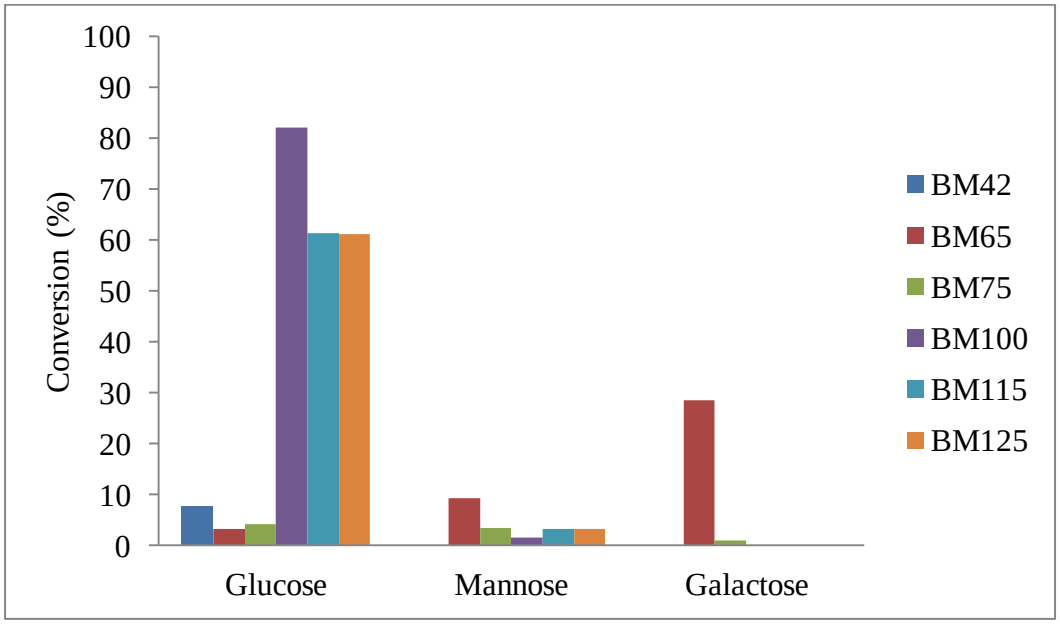

Figure 4.9 : Conversion ratios for glucose, mannose and galactose.

Next results are steam explosion results. BM75 is used for steam explosion. In steam explosion experiment, S1 and S9 have same conditions so S19 which is shown in Figure 4.10 is average of S1 and S9.

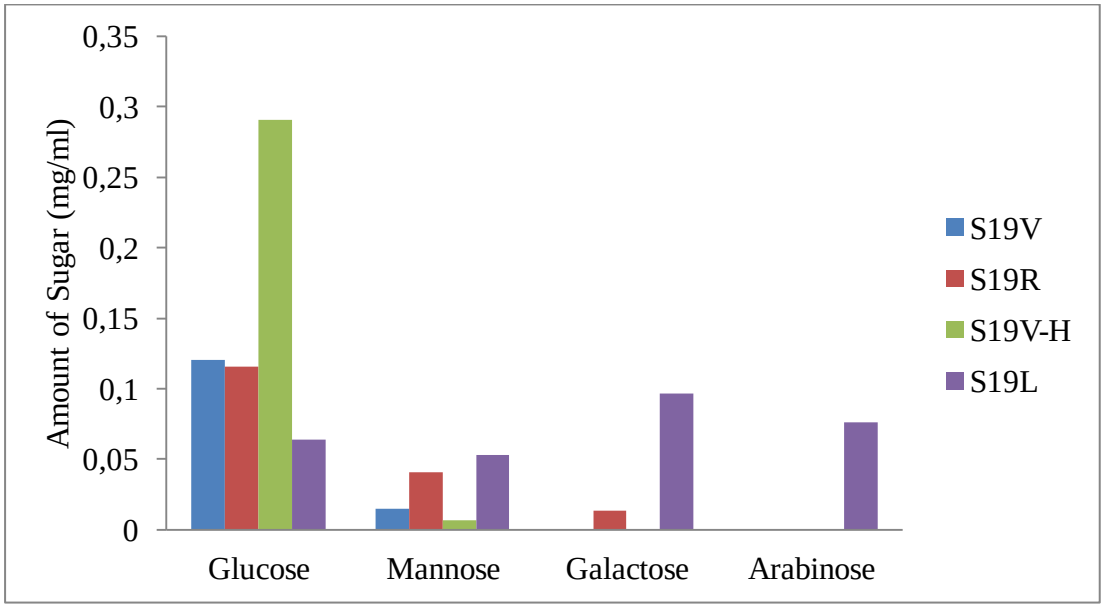

Figure 4.10 : Average of S1 and S9.

According to Figure 4.10, S19V-H has most glucose conversion than others and sample which was collected from vessel has more glucose than sample which was

collected from reactor. On the other hand, S19L has more mannose, galactose and arabinose. Also S2, S3 and S7 are in same conditions. Figure 4.11 is shown that average of sugar determination results of these experiments. S237 shows average values of S2, S3 and S7.

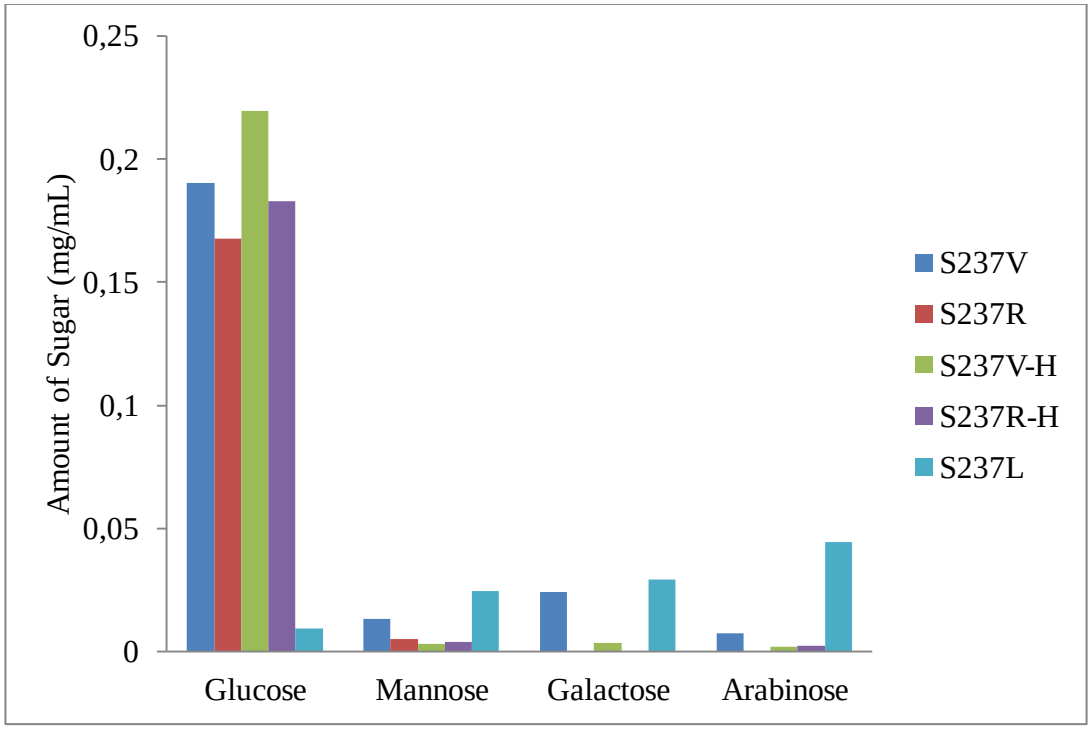

Figure 4.11 : Average of sugar determination of S2, S3 and S7.

As it is seen in Figure 4.11 that, samples which were collected from vessel have more glucose than samples which were collected from reactor.

The one of the variable parameter is nitrogen pressure. S6 and S10, S237 and S19 have same parameters without nitrogen addition. S6 and S10 are 8 grams and fully opened valve. However, S6 has 64 psig nitrogen and S10 is 0. On the other hand, S237 and S19 are 4 grams and fully opened valve. However, S237 has 64 psig nitrogen pressure and S19 has 0. Figure 4.12 and Figure 4.13 show that nitrogen affects on sugar determination.

According to Figure 4.12, glucose content of S6 is more than S10. Nitrogen pressure of S6 is 64 psig; however S10 has 0. Glucose content of S237 is higher than S29. As it is same as Figure 4.12, increasing nitrogen pressure causes increasing glucose obtaining.

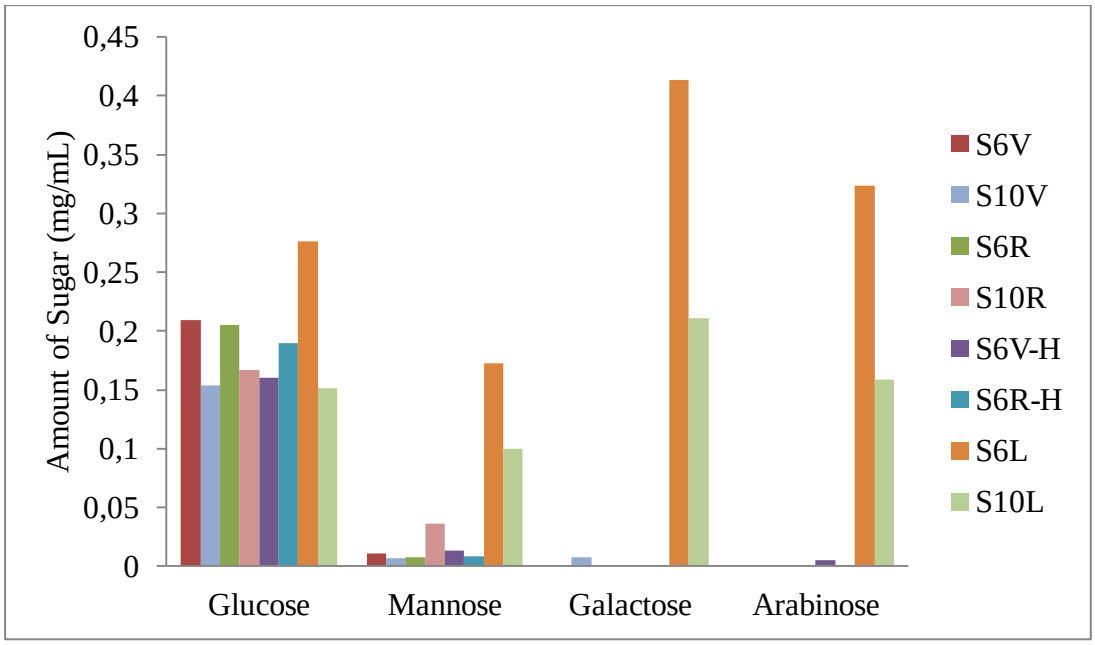

Figure 4.12 : Sugar determination of S6 and S10.

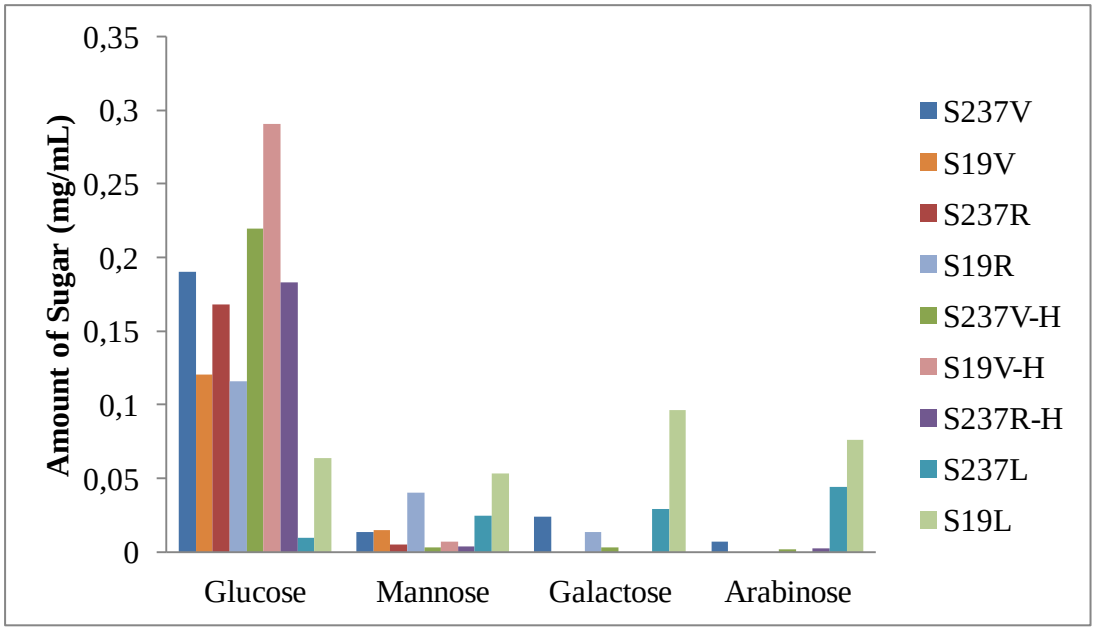

Figure 4.13 : Sugar determination of S237 and S19.

Other variable parameter is valve condition. In this experiment there are two condition of valve. One is fully open and other is 360° open. For this experiment, S237 and S5 are compared and they are 8 g, nitrogen pressure is 64 psig. S6 and S8 are compared and they are 4 g, nitrogen pressure is 64 psig. S237 and S6 valves are fully open but S5 and S8 valves are 360° open. Figure 4.14 and Figure 4.15 show that how valve condition effect sugar conversion.

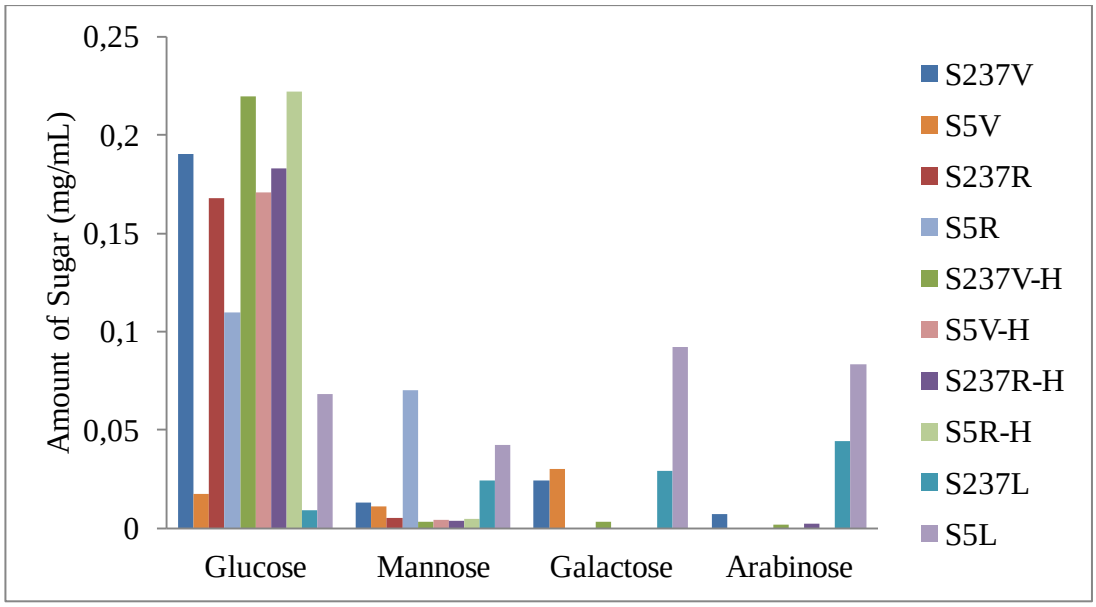

Figure 4.14 : Valve condition effect on sugar conversion (S237 and S5).

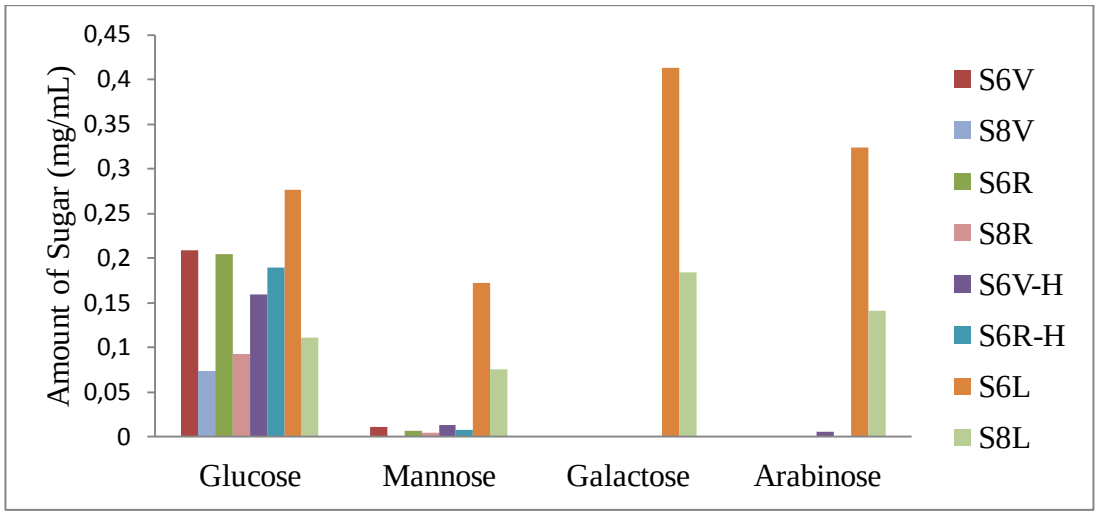

Figure 4.15 : Valve condition effect on sugar conversion (S6 and S8).

In Figure 4.14, glucose content of S237 is higher than S5 except liquid part and reactor part. S237 is fully opened valve and S5 is 360° opened valve. As it is seen in Figure 4.15, glucose content of S6 is higher than S8. According to results of Figure 4.14 and Figure 4.15, fully opened valve is higher glucose conversion than 360° opened valve.

The other variable parameter is amount of sawdust. In this experiment 2 different amount of sawdust is used. These are 4 and 8 g of sawdust. S6 and S237 are compared and they are 64 psig nitrogen added and fully open, S19 and S10 are compared and they are 0 psig nitrogen added and fully open. S237 and S19 are 4 g,

S6 and S10 are 8 g. Figure 4.16 and Figure 4.17 show that how amount of sawdust effect on sugar conversion.

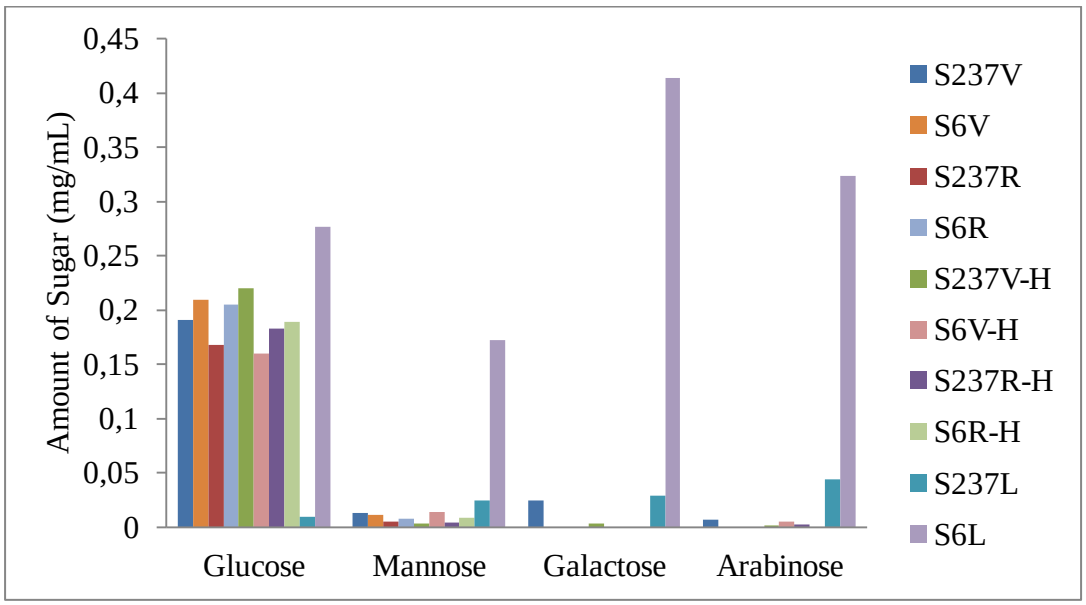

Figure 4.16 : Amount of sawdust affect on sugar conversion (S6 and S237).

As it is seen in Figure 4.16 that, values of S6 are greater that values of S237. The difference between S6 and S237 is quantity of sawdust which was used in experiment. Amount of sawdust at S6 is 8 g, at S237 is 4g. Referring to Figure 4.17, sugar content of S10 is higher than S19. According to Figure 4.16 and Figure 4.17, in experiments, 8 g of samples were converted to sugar more than 4 g of samples.

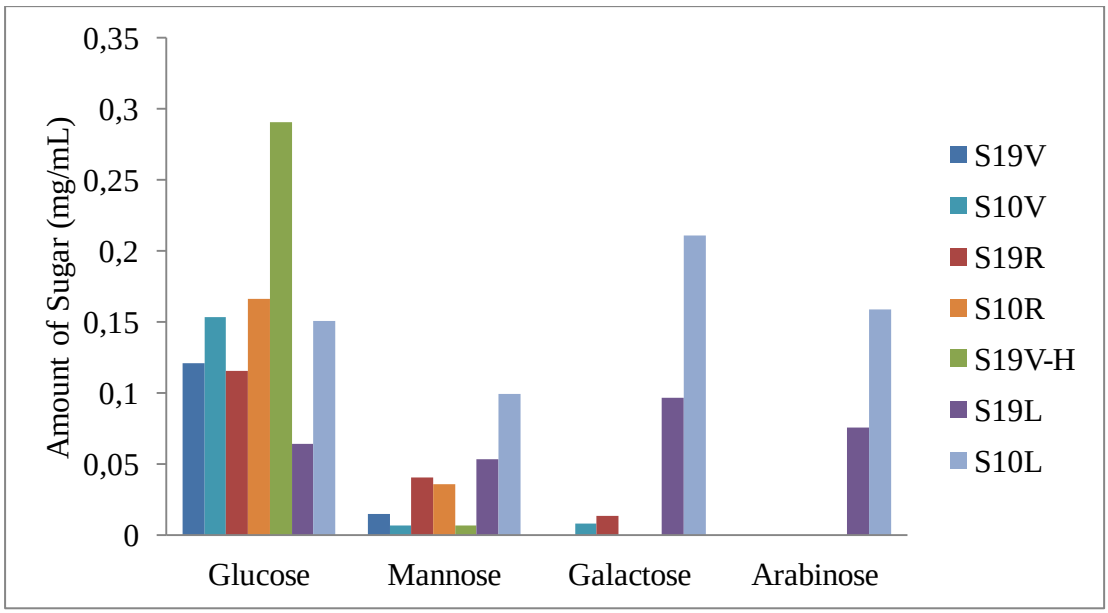

Figure 4.17 : Amount of sawdust affect on sugar conversion (S19 and S10).

CONCLUSIONS

In this chapter, the current study will be summarized and derived results will be clarified. Moreover, some recommendations, outlook and further aspects are mentioned.

In this study, physical properties of pine wood are defined. Then ball mill and steam explosion pretreatment methods are applied. Cellulase enzyme activity and sugar content are measured.

First of all, pine timber was taken and shaved. Pine shavings were sieved and moisture, ash and lignin analyzed were done. 35 mesh sized particles were used for ball mill pretreatment because of being highest amount. Ball mill pretreatments were operated at 2,4,8,12,16 and 24 h. Optimum working conditions were chosed as

- Ball number : 25 balls
- Ball diameter : 12 mm
- Speed : 200 rpm
- Sample Amount : 10 g
- Pretreatment time : 8 hours

The reason of choosing 8 hour is, according Table 4.3, after 8 hours, the amount of 75 mesh sized particle are highest. The reasons of chosing 75 mesh size are cellulose activity of 75 mesh sized particles were not high as much as 100, 115 and 125 mesh sized particles, obtaining 75 mesh sized particles is easier than riner particles due to retention time and energy consumption and finer particles could be act as dust and had motion with air so it causes lost of feedstokcs. After ball mill pretreatment steam explosion method were applied. The variables of steam explosion method are quantity of sawdust, temperature, nitrogen pressure and releasing time (valve condition). In this study, 190°C and 240°C was applied, however samples was burnt at 240°C. Then 190°C were chosed for steam explosion. 4 g and 8 g samples, 0 and 64 psig nitrogen pressure were tested in this pretreatment. Valve condition is defined as

releasing time. The releasing time of fully open valve was nearly 20-25 sec and 360° open valve was 45-60 sec. According to applying conditions in this study, highest conversion was found depends on HPLC results at;

- Temperature : 190°C
- Quantitiy of Sawdust : 8 g
- Nitrogen Pressure : 64 psig
- Retention Time : 10 min
- Releasing Time : 22 sec
- Valve Condition : Fully open

The next step of this project, pretreated samples will be fermentated and ethanol yield will be measured. Then life cycle assessment should be calculated. Feasibility of bioethanol should be doing. These experiments should be redone for another woody biomass which includes more cellulose content and also different enzymatic catalyst could be used.

In Turkey, agricultural potential is available for bioethanol production. Bioethanol feedstocks which are sugar beets, molasses, wheat and corn harvested in Turkey. At present, first generation bioethanol is produced from corn, wheat and sugar beets. Second generation bioethanol shoul be produced from lignocellulosic materials. However, lignocellulosic potential of Turkey is not defined well. It should be defined for development of lignocellulosic alcohol production.

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CURRICULUM VITAE

Name Surname: Merve Nazlı ERDÖNMEZ BORAND

Place and Date of Birth: Bursa, 30/01/1986

Address: Vişnezade Mah. Armağan Sok. Uğur Apt No:4/11, Beşiktaş / İstanbul

E-Mail: nazlierdonmez@msn.com; nazli.erdonmez@yalova.edu.tr

B.Sc.: Istanbul Technical University, Chemical Engineering Department (2009)

Professional Experience and Rewards:

YOK (The Council of Higher Education) - 6 months Abroad Master Research Scholarship, University of Idaho, Biological and Agricultural Department, Visiting Scholar, Idaho, USA, 2010 - 2011.

PUBLICATIONS/PRESENTATIONS ON THE THESIS

Erdönmez M., N., İşler A., Karaosmanoğlu F., "The Current Situation of Engine Fuels in Turkey", *American Society of Agricultural and Biological Engineering (ASABE) Bridges to Sustainable Agriculture Conference*, September 9-11, 2010 Lethbridge, Canada.

Jain T., Deis B., Erdönmez M. N., Van Gerpen J., Feris K., ''Enzymatic Conversion of LignocellulosicBiomass to Sugars'', *ASABE (American Society of Agricultural and Biological Engineering) Bridges to Sustainable Agriculture Conference*, September 9-11, 2010, Lethbridge, Canada.